

CROSS-PHYLA INVESTIGATION INTO THE EFFECTS OF APPLIED WEAK-
INTENSITY ELECTROMAGNETIC FIELDS

by

Ryan C. Burke

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APPROVED/APPROUVÉ

Thesis Examiners/Examineurs de thèse:

Dr. Michael Persinger

(Supervisor/Directeur(trice) de thèse)

Dr. Peter Ryser

(Committee member/Membre du comité)

Dr. Suzanne Lamothe

(Committee member/Membre du comité)

Dr. Michael Levin

(External Examiner/Examineur externe)

Approved for the Faculty of Graduate Studies

Approuvé pour la Faculté des études supérieures

Dr. David Lesbarrères

M. David Lesbarrères

Acting Dean, Faculty of Graduate Studies

Doyen intérimaire, Faculté des études supérieures

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Abstract

This series of studies investigated the effects of applied, low-intensity electromagnetic fields on the behaviour of several species. To cover a range of species; the eusocial harvester ants (*Pogonomyrmex sp.*), solitary orb-weaving spiders, and aquatic planarian (*Dugesia tigrina*) were examined for behavioural consequences associated with applied electromagnetic fields. An additional component examined these effects on various volumes of water. In all species examined, significant behavioural consequences were observed. Intensities of the used fields ranged from nanotesla to millitesla, and their patterns included a fixed-pattern 60 Hz field, and a more complex-patterned field. A separate component also analyzed the effects of light and polarity, where additional effects were evident. For the experiments with the harvester ants, significant changes in tunneling behavior were observed; for the spiders, significant changes in the structure of the web were observed; for the planarian, significant effects on t-maze arm selection occurred; and for water, significant changes in pH were detected.

Keywords:

Harvester ant, tunneling, orb-weaving spider, web structure, planarian, t-maze, arm selection, water, pH, electromagnetic, coin magnets, polarity, light, BurstX, 60Hz, intensity, pattern

Co-authorship Statement

Papers included in this thesis document are co-authored by Dr. M. A. Persinger.

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Chapter 1: Introduction

1.1 Introduction

The 1970's have been defined as the beginning of the information age, a period in time where technological advancements began drastically changing the shape of society. The rapid move to digitize information produced a massive thrust in technology-based and telecommunications-based sectors of the economy. This ushered in incredible advances such as cellular phones, the Internet, and ultimately our dependence on technology. According to the 2013 estimates from the International Communication Union, there are approximately 96 mobile cellular subscriptions for every one hundred people in the world, and 39 percent of the world's population are connected to the internet (International Communication Union, 2013).

Often when we think of our effects on the planet, deforestation, pollution, and resource-depletion are commonly discussed themes. Yet, in a digital world where electronic information is the new petroleum and electromagnetic fields are the by-product, a potential global electromagnetic impact is rarely considered. The average person consumes over 3,000 kWh of electricity per year (UN data, 2011). That works out to an equivalent of 10.8 billion joules of energy per person per year, or 75.6 exajoules ($7.56 \times 10^{19}\text{J}$) for the entire population. To put that into perspective, we consume more energy annually than the explosive energy in one hundred replicas of the world's largest ever tested nuclear bomb (Tsar bomba, 2014). For perspective the power density distributed over the surface of the planet would be equivalent to about 1 lux if it were

within the visible wavelength. In other words the background glow would be similar to that of a full moon.

It's not necessarily the amount of electricity consumed annually that is relevant to this study, rather it is directly related to the annual production of human-made electromagnetic radiation. This electromagnetic energy is emitted from our homes, offices, libraries, vehicles, stores, personal communication devices in a multitude of patterns and intensities. One consequence of our tremendous technological advances is that we and the rest of the planet are continuously immersed in these electromagnetic signals.

Social terrestrial (harvester ants, *Pogonomyrmex sp.*), solitary terrestrial (orb-weaving spiders, *Araneidae sp.*), and aquatic (planarian, *Dugesia tigrina*) invertebrates were selected to measure potential effects of applied electromagnetic fields on a wide array of species under different behavioural paradigms. An additional chapter reports the results of the assessment of similar electromagnetic effects upon the pH of water, and will serve to provide a potential platform for which large-scale biological effects could result. Two important questions will be addressed in this study: First, is there evidence that low-intensity electromagnetic fields (10^{-9} - 10^{-6} Tesla range) influence the behaviour of focus species in this study? And second, if behavioural effects are observed, are they dependent on the polarity or pattern of field presentation?

1.2 Bioelectromagnetism: Theory

One would expect that if magnetoreception is present among the focus species for this investigation, there would exist an inherent evolutionary advantage for this sensory

system. The importance of chemoreception in harvester ants, mechanoreception in orb-weaving spiders, and photoreception in planarian is very well established. Each system offers an advantage to their respective species. For example, chemical reception allows for communication among members in an ant colony, mechanical reception is important for detecting small disturbances in webs of orb-weaving spiders, and photoreception plays an important role in planarian navigation.

What then would be a potential advantage in the evolution of a sensory system designed to detect changes in electromagnetic fields? Fundamentally, any organism requires access to food for energy and shelter to protect the organism from becoming another's food. Therefore, one would expect that magnetoreception, like any other sense, would increase the organisms chance at accessing either food or shelter. Although no research could be found to answer for this specific perspective for species employed for this investigation, other research has demonstrated relationships between orientation and navigation with the Earth's magnetic field. Others have demonstrated a relationship between the geomagnetic field and climate patterns. The following sections will summarize literature pertaining to the geomagnetic influence on orientation/navigation of several species and on weather patterns.

1.3 Geomagnetic influence on orientation/navigation cues

An abundance of literature has demonstrated that the geomagnetic field plays a role in the orientation and navigation of species ranging from bacteria to humans. Magnetoreception in bacteria was accidentally discovered when a researcher studying marine sediment for *Spirochaeta plicatilis* noticed that a group of microorganisms in the sample tended to migrate to one side of the slide under microscopic examination.

After rotating the slide by 180 degrees, these same bacteria would migrate toward their initial geographic direction. This effect was confirmed after the same observations were made under different lighting conditions to rule out a phototactic response. Moreover, it was determined that direction of migration of these organisms could be modified in the presence of a magnet (Blakemore, 1975). In the same study, he described a characteristic feature of magnetotactic bacteria was the inclusion of intra-cellular chains of 5-10 crystal-like particles predominantly made of iron evident when imaged using electron microscopy. In the presence of an externally applied magnet, chains of cells formed and acted as one dipole in response to changes in the position of the magnet.

Later Blakemore published a review on the magnetotactic behaviour of bacteria demonstrating a unique feature. Magnetoreceptive bacteria in the northern hemisphere demonstrate magnetic north-seeking behaviour (Blakemore, 1975; Moench & Konetzka, 1978; Blakemore & Frankel, 1981). Those in the southern hemisphere demonstrated the exact opposite (Blakemore et al., 1980; Kirschvink, 1980; Blakemore & Frankel, 1981). Although no research on the effects of magnetic fields could be found with spiders, one species of planarian (*Dugesia dorotocephala*); and two species of ant (*Solenopsis invicta*, *Formica rufa*) have demonstrated the utilization of geomagnetic cues for orientation (Brown, 1962; Anderson & Van der Meer, 1993; Camlitepe & Stradling, 1995).

One of the pioneers who investigated the responses of invertebrates to geophysical variables was Frank A. Brown, Jr. He not only investigated geomagnetic effects, but also solar and lunar effects on several invertebrates. In follow-up to a series of papers demonstrating the marine mud snail, *Nassarius obsoletus*, was capable of

detecting small changes in the horizontal component of a magnetic field (Brown et al., 1960; Brown, Webb, and Bennet, 1960; Barnwell and Brown, 1961), Brown (1962) performed similar tests on the planarian, *Dugesia dorotocephala*, to see if the geophysical effects were perhaps widely distributed across many species.

The studies involved monitoring the path of several thousand (N=3493) planarian for mean daily deviations in degrees from their initial path over the course of November, 1960 until April 1962. The angular deviation in trajectory for the first inch of free swim was measured, with the apparatus geographically oriented. A significant lunar relationship was evident with maximum counter-clockwise turning at the time of the new moon. The maximum clockwise turning occurred around the time of the full moon. Interestingly as shown Figure 1.1, this effect dissipated in the summer months and became extremely evident in the winter months (Brown, 1962; Figure 4). Moreover, the application of a 4 Gauss magnetic field in the north-south direction completely abolished this cycle, suggesting that magnetoreception does influence orientation in this species.

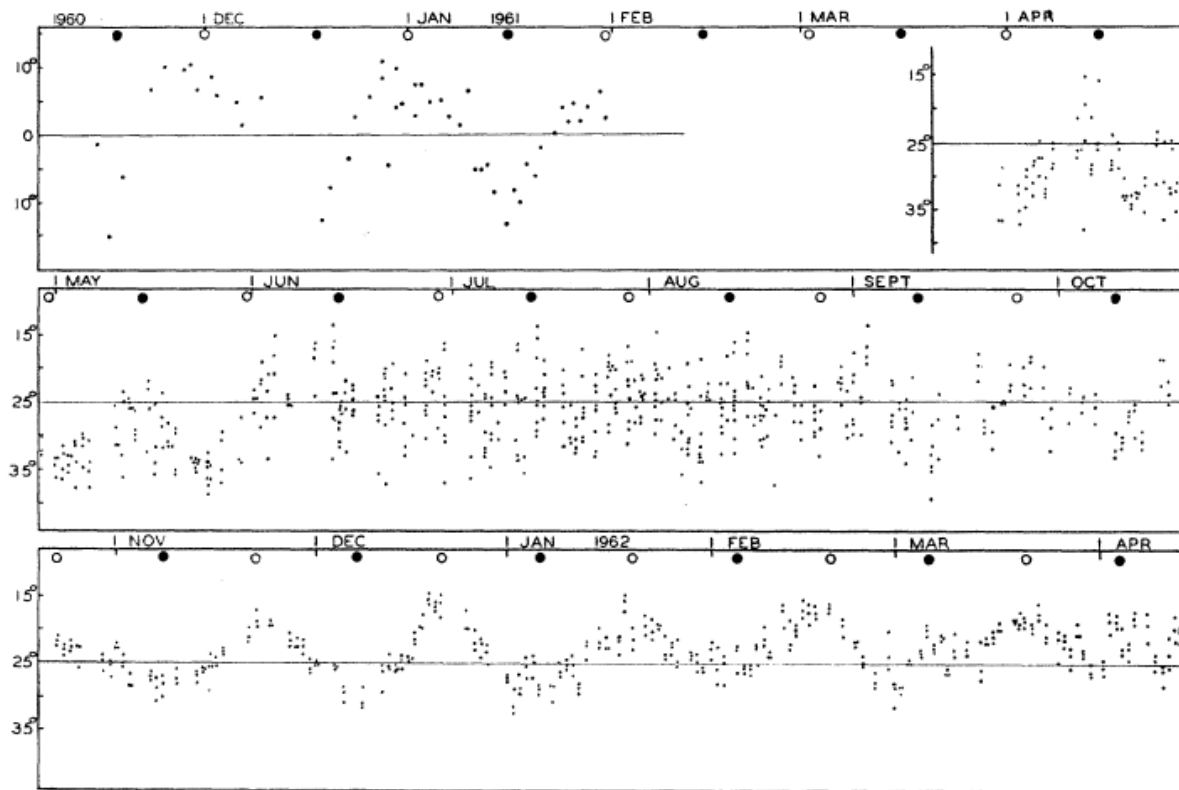


Figure 1.1 (Courtesy of Brown, 1962, figure 4); figure demonstrates the monthly lunar influence on the orientation of the *Dugesia dorotocephala*. Prior to April, 1961 each dot represents the average of 45-140 paths; whereas, each dot after this date represents the mean of 15 paths. The dark and light circles represent the new and full moon, respectively.

In the study conducted by Anderson and Van der Meer (1993) colonies of ants were investigated to discern if the geomagnetic field influenced their navigation to a food source. Four conditions were created; two where their geomagnetic environments remained constant, and two where they were reversed subsequent to an acclimatization period and adding a food source. The four conditions are as follows: Reverse/Reverse, Normal/Normal, Reverse/Normal, and Normal/Reverse, where normal refers to background geomagnetic conditions and reverse refers to a 180 degree reversal of the

geomagnetic field using a solenoid. Results from this experiment demonstrated a significant impairment for the time required to form a trail to the food source in both of the reversed field conditions when compared to the non-reversed conditions [$p < 0.001$ for all]. The average difference in time was almost doubled, being approximately 30 minutes for the reversed groups and approximately 15 minutes for the non-reversed groups. The change in direction appeared to be the critical factor because there were no significant difference between either of the non-reversed conditions [$p = .804$]. Similar results were obtained in the Camlitepe and Stradling (1995) study in which the *Formica rufa* species was investigated. However, the rotation of the field was 90 degrees for this experiment.

Other invertebrates such as honeybees and spiny lobsters have also demonstrated magnetic orientation with respect to the geomagnetic field. One study demonstrated how large variability in geomagnetic activity reduced honeybees ability to accurately indicate the location of food source through the bee dance (Lindauer & Martin, 1968). Further studies with bees have demonstrated that geomagnetic cues are utilized for comb construction (Martin & Lindauer, 1972) and for spatial learning (Frier et al., 1996). Several experiments have further demonstrated that honeybees can be conditioned to respond to very small changes in the geomagnetic field intensity (Walker & Bitterman, 1985; Walker & Bitterman, 1989;) with a threshold in the range 26-2600nT (Walker & Bitterman, 1989).

Research with spiny lobsters (Lohman et al., 1995; Boles & Lohman, 2003) has provided valuable insight into a potential single component of the geomagnetic field influencing orientation. When comparing controls to those who had an externally

applied reversal of electromagnetic field, similar in intensity to the geomagnetic background, in either the vertical or horizontal components differences were observed. Whereas the control and reversed vertical groups did not significantly differ from one another, deviating from their course by an average of 10 and 3 degrees, respectively; those in the reversed horizontal groups deviated on average 183 degrees. This provided evidence that the horizontal component, at least for the spiny lobster, was responsible for the observed behavioural effects.

In addition to the above, research demonstrating a geomagnetic role in navigation has also been confirmed in reptiles and amphibians (Phillips, 1986; Lohman, 1991; Salmon & Lohman, 1993), rodents (Marhold, Wiltschko & Burda, 1997; Thaleau et al., 2006), birds (Alerstam, 1987; Fransson et al., 2001; Wiltschko & Wiltschko, 2005; Thaleau et al., 2006;), and humans (Kirschvink et al., 1992; Finney, 1995; Foley et al., 2011). When taken together, vast amounts of evidence demonstrate the importance the geomagnetic field plays on the navigation patterns of potentially all species.

1.4 Geomagnetic influence on weather patterns

Alternatively to a navigation purpose, magnetoreception could potentially be an important evolutionary adaptation for detecting imminent changes in weather. Long term associations between the geomagnetic field and global temperature have been explored (Bucha, 1976; Persinger, 2010). The effects of geomagnetic storms on pressure and temperature have been investigated on a much shorter time scale for over fifty years. The first study involved the jet stream patterns at the 300 mb level

(approximately 9 300 meters above sea level) over the Alaskan-Aleutian area for the winters of 1956-1957 and 1957-1958. This study was the first to demonstrate a time-lagged geomagnetic effect on pressure systems. This effect demonstrated that significant pressure troughs occurred 3 days following geomagnetic storms (MacDonald & Woodbridge, 1959). This same time-lagged effect was demonstrated at the 500 mb level (5 500 meters above sea level) whereby significant pressure troughs were associated with geomagnetic storms 3 days prior (Twitchell, 1963).

Since solar activity and geomagnetic activity are strongly correlated over time, one argument could suggest the Sun may be influencing these changes. Woodbridge (1971) published results that highlighted same phenomenon over periods of solar maxima and minima. It was found that the aforementioned relationships were evident during both times. Further contributions were made for different regions and time periods that confirmed other research demonstrating the development of significant pressure troughs roughly three days following a geomagnetic storm (Roberts & Olson 1971; Roberts & Olson, 1973).

The Geophysical Institute, Academy of Science in Czech Republic extended previous research to include the full northern hemisphere over longer time periods (1952-1996). In separate publications this team demonstrated that geomagnetic activity was more closely related to the observed temperature deviations than was solar activity (Pycha et al., 1992; Bochnicek & Pycha, 1994; Bochnicek et al., 1996). In a later publication, this group looked at climate effects at sea level rather than atmospheric conditions, and the results are summarized in Table 1.1 below (Bochnicek et al., 1999).

Unique relationships are observed between the intensity of the geomagnetic field and weather across the northern hemisphere.

High ΣK_p	Low ΣK_p
Icelandic low pressure deepens	Icelandic low pressure fills
Extensive negative pressure deviations	Isolated areas of positive and negative pressure deviations
Warm air front over USA, Europe, Asia	Cold air front over Europe
Cold air over Greenland	Warm air over Greenland

Table 1.1- Summary of results from Bochnicek et al., 1999, which demonstrated significant pressure and temperature changes associated with high geomagnetic activity. K_p refers to the planetary K-index, which is a measure of the horizontal component of the geomagnetic field.

In addition to benefiting from knowledge of approaching weather systems, some species may also benefit from seasonal cues which could guide their behaviour. Courtesy of Sodankyla Geophysical Observatory, nearly 100 years of daily geomagnetic data were averaged into months, and the results are illustrated in Figure 1.2 below. It's clear that seasonal differences in the average geomagnetic intensity are persistent across time.

Taken together, geomagnetic activity clearly has an influence on weather systems as demonstrated from sea level to the edges of Earth's atmosphere. From an evolutionary perspective, it is quite plausible that magnetoreception emerged as a sensory system that offered a small window of time to adjust to seasonal variation or impending weather changes.

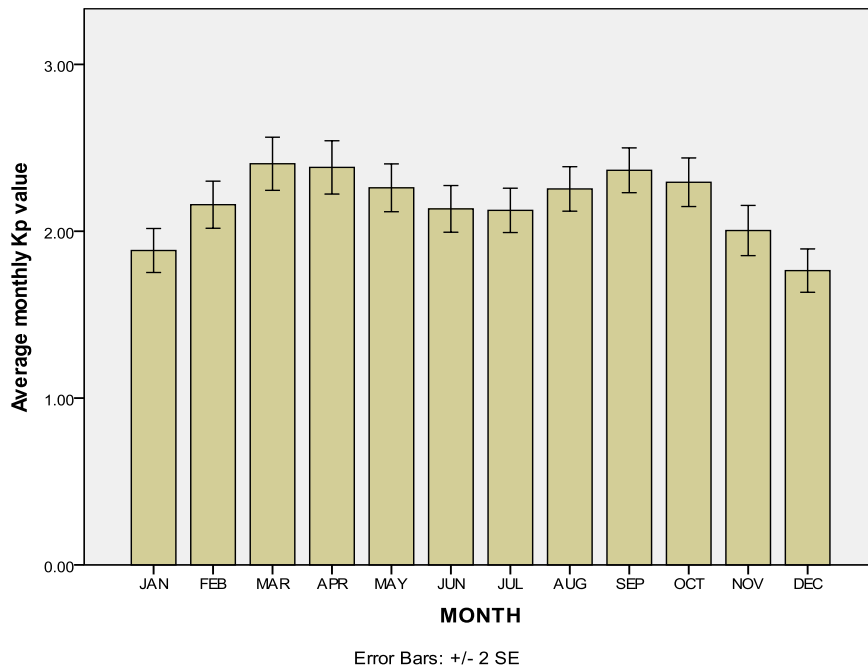


Figure 1.2- Averaged monthly values for 100 years of the planetary K-index demonstrating a persistent seasonal variation. Data courtesy of Sodankyla Geophysical Observatory.

1.5 Physiological candidates for magnetoreception

Despite abundant research demonstrating the geomagnetic field influence on orientation and navigation across a variety of species, no structure to this date has been causally implicated in magnetoreception. With that stated, two strong candidates appear to be consistent among many magnetoreceptive species and may describe different types of magnetoreception. The first of which are biogenic magnetite particles that are involved in a light-independent type of magnetoreception. The second type is proposed to be a light-dependent mechanism mediated by cryptochrome proteins. The following

sections will summarize literature on these two independent types of magnetoreception, which have been observed in many species.

1.6 Biogenic magnetite

Biogenic magnetite particles have been observed in many species. However, their presence was confirmed following an accidental finding when aquatic bacteria were observed navigating toward the north pole on the slide they were situated (Blakemore, 1975). Subsequently these bacteria were observed under transmission electron microscopy which revealed the presence of linear chains of crystal-like particles. These particles were later determined to consist primarily of iron (Figure 1.3).

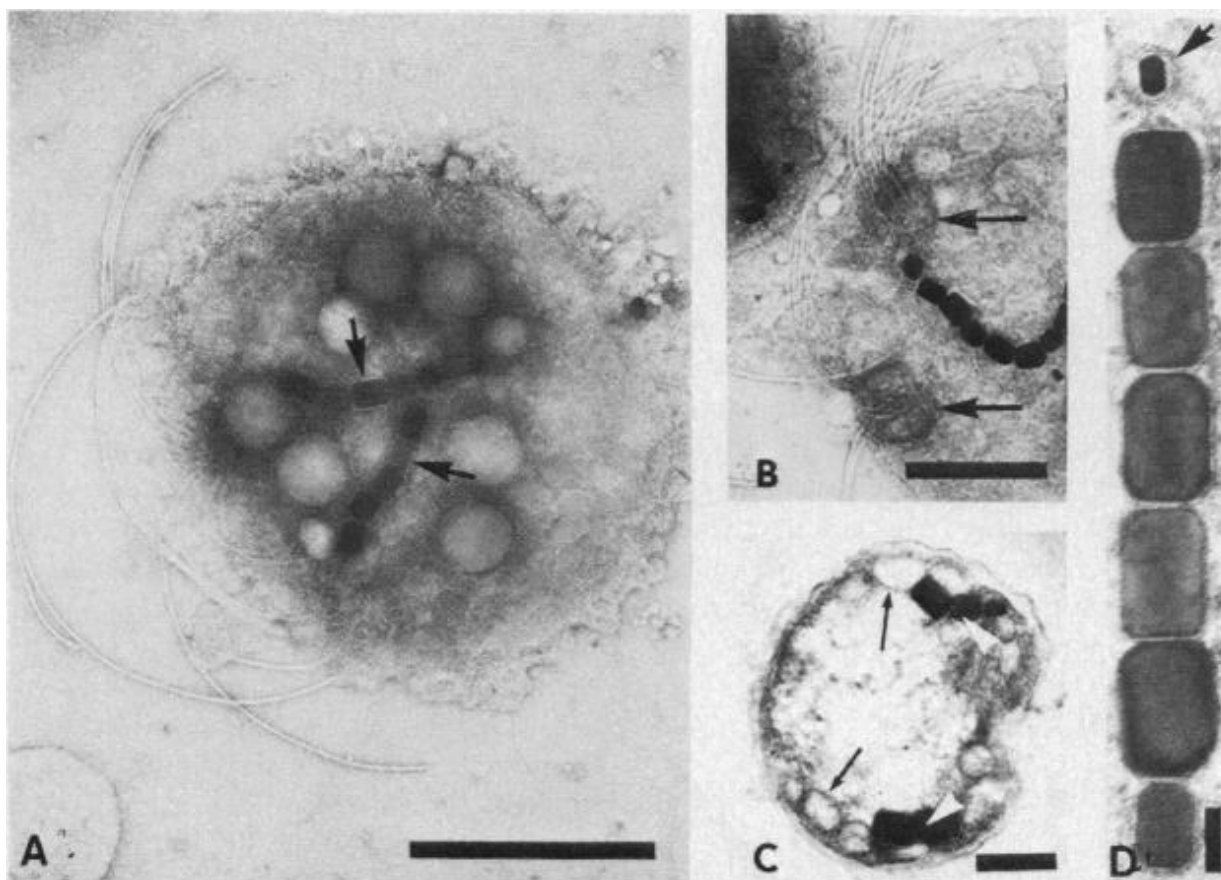


Figure 1.3 a,b,c- (Courtesy of Blakemore, 1975, figure 4); Multiple examples of the intracellular, biogenic magnetite within the bacteria investigated. In figures "A" and "D" the arrows point to the ferrous oxide particles; whereas, in figures "B" and "C" they are pointing to other cellular components.

The presence of biogenic magnetite particles has also been discovered in two species of ants: the *Pachycondyla marginata* (Acosta-Avalos et al., 1999; Oliveira et al., 2010) and the *Solenopsis subnitida* (Abracado et al., 2005). In all three studies, ants were separated into head, abdomen, and thorax sections for analysis of ferrous oxide content. Although different methods were used in each, all three demonstrate the presence of iron-dominant compounds in the head and abdomen. The study conducted

by Oliveira and colleagues (2010) used embedded tissues in order to account for the potential confound of soil inclusions that cannot be ruled out with certainty in the other two studies. Specifically, in this study the highest amount of magnetic material was determined to be in specific joints in the ants' antennae that are in very close proximity to sensory processes (Figure 1.4).

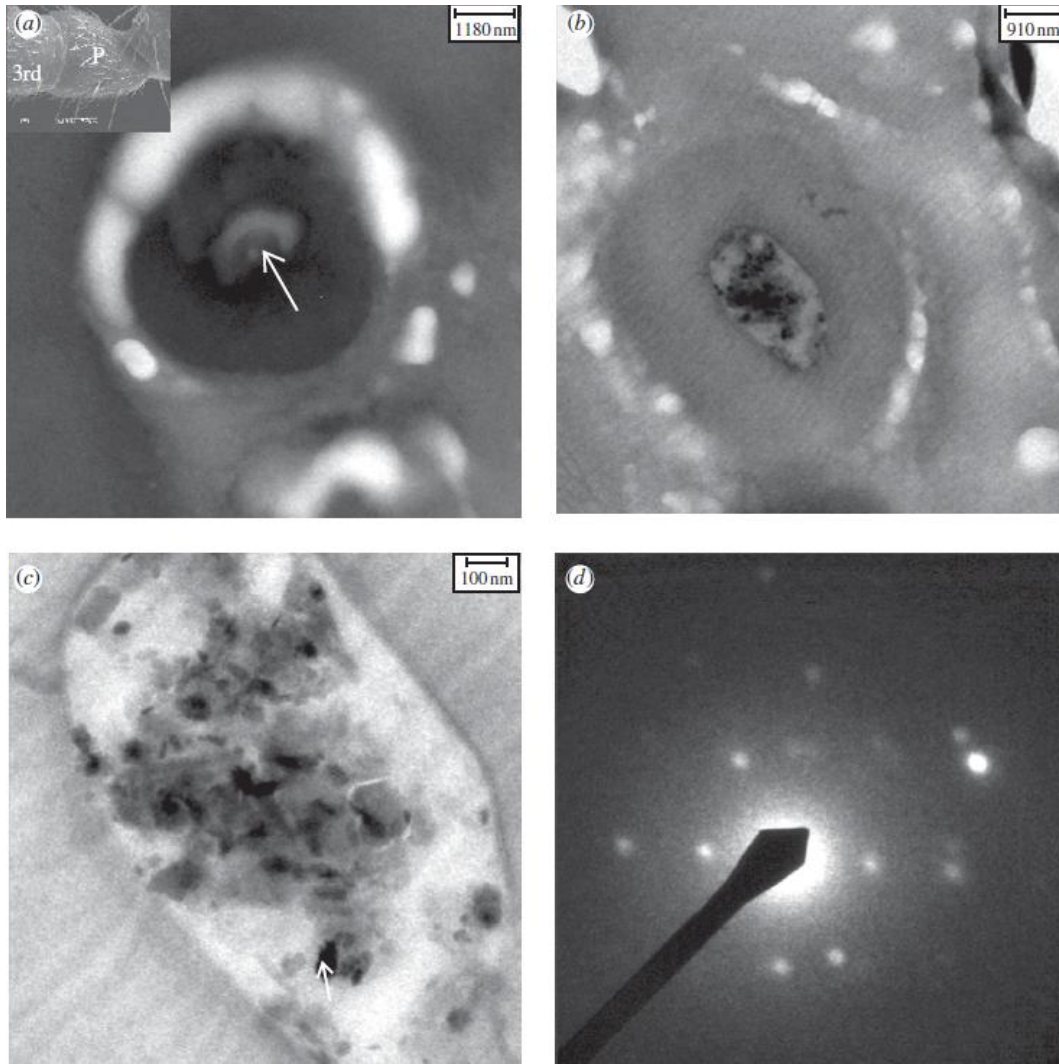


Figure 1.4(Courtesy of Oliveira et al., 2010, figure 4 a-d); Figure "A" is a transverse section of a cuticular knob cut at the joint between the 3rd segment of the antenna and the pedicel as demonstrated in the inset, with the arrow pointing at a sensory process; Figure "B" is another transverse section of a cuticle with the presence of ferrous oxide compounds; Figure "C" is a higher power image of "B", with the arrow pointing at the magnetic crystals; Figure "D" is the x-ray diffraction profile of one of the crystals which allowed for its identification as goethite.

The presence of intracellular biogenic magnetic particles has additionally been located in molluscs (Lowenstam, 1962), several fish species (Vilches-Troya, Dunn, and O'Leary, 1984; Mann et al., 1988), invertebrates including termites (Maher, 1998) and honey bees (Kuterbach et al., 1982), homing pigeons (Hanzlik et al., 2000; Winklhofer et al., 2001; Fleissner et al., 2003), and humans (Kirschvink, Kobayashi-Kirschvink, and Woodford, 1992; Schultheiss-Grassi, Heller, and Dobson, 1997; Schultheiss-Grassi and Dobson, 1999).

The proposed mechanism of action has been explored in depth by many authors (Frankel, 1984; Winklhofer, 2009; Winklhofer and Kirschvink, 2010; Linnebach, 2012). When the magnetite chains are exposed to a magnetic field, like the geomagnetic field, they act as a dipole and preferentially orient parallel with the magnetic flux lines. Any deviations away from parallel would result in a torque acting on the magnetite chains, and potentially cause changes in membrane potential of nearby sensory neurons through connections with mechanoreceptors.

1.7 Cryptochrome

Cryptochrome-mediated magnetoreception has been extensively studied in the fruit fly (Gegear et al., 2008; Yoshii, Ahmad, and Helfrich-Forster, 2009), the eastern red-spotted newt (Phillips and Borland, 1992 a,b; Deutschlander, Borland, and Phillips, 1999), and several species of birds (Wiltschko et al., 1993; Wiltschko et al., 2010). Cryptochrome is a photopigment molecule that responds to light in the UVA/blue-light

spectrum (Gressel, 1979; Hsu et al., 1996; Berndt et al., 2007), and is proposed to mediate light-dependent magnetoreception.

In the study conducted by Gegear and colleagues (2008), the Canton-s strain of *Drosophila* was utilized because they demonstrated a reliable preference for a magnetic field once trained under full spectrum white light (approx. 300 nm-700 nm). As outlined in Figure 1.5 below the *Drosophila*'s magnetic compass was abolished when cryptochrome-sensitive wavelengths were blocked and returned when they were not. In further support for cryptochrome proteins as the light-dependent magnetoreceptor, when the gene coding for these proteins was blocked so too was the magnetoreception.

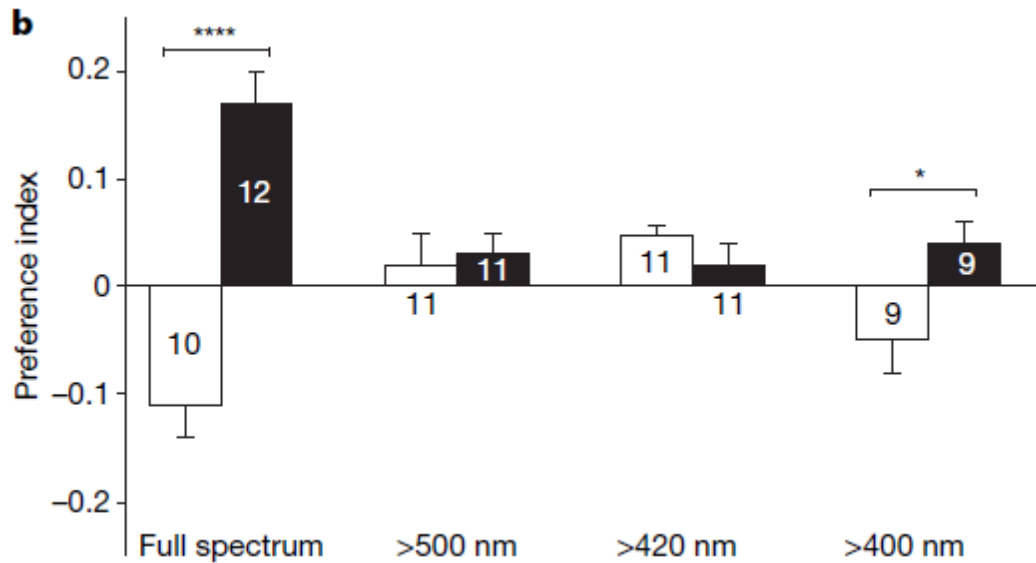


Figure 1.5- (Courtesy of Gegear et al., 2008, figure 2b); this figure demonstrates the inability to respond to magnetic cues when cryptochrome-sensitive wavelengths are blocked; **** $p < 0.001$; * $p < 0.05$; the numbers in the bars indicate the number of groups tested (100-150 per group). For comparison the white bars represent the naive group and the black bars represent the group with the paired magnetic field association to a sucrose reward. The preference index value is calculated using the equation, $(PM - 0.5)/(PM + 0.5) - (2PM \times 0.5)$, where PM is the proportion of flies on the magnetic field side of the t-port.

Two studies by Philips and Borland (1992 a,b) investigated the effects of various wavelengths of light upon the magnetic compass response in the eastern red-spotted newt. Male newts, *Notophthalmus viridescens*, were trained to give a consistent magnetic compass response under natural light conditions. When brought into an indoor lab

environment those tested under a full spectrum white light were significantly oriented in the same magnetic direction as they were tested. Newts tested under 400 nm and 450 nm conditions responded similar to those in the natural and full spectrum conditions. However, those tested under 500 nm, 550 nm, and 600 nm conditions were oriented 90 degrees counter clockwise to the other conditions.

To determine whether there was a motivational influence or a magnetic compass influence newts were subsequently trained under a 500 nm filter. When tested as before, newts in the 500 nm, 550 nm, and 600 nm conditions were oriented in the direction in which they were trained; however, those tested under the full spectrum condition oriented 90 degrees clockwise to the trained direction (Figure 1.6). When tested under >715 nm wavelengths the newts magnetic compass was completely abolished.

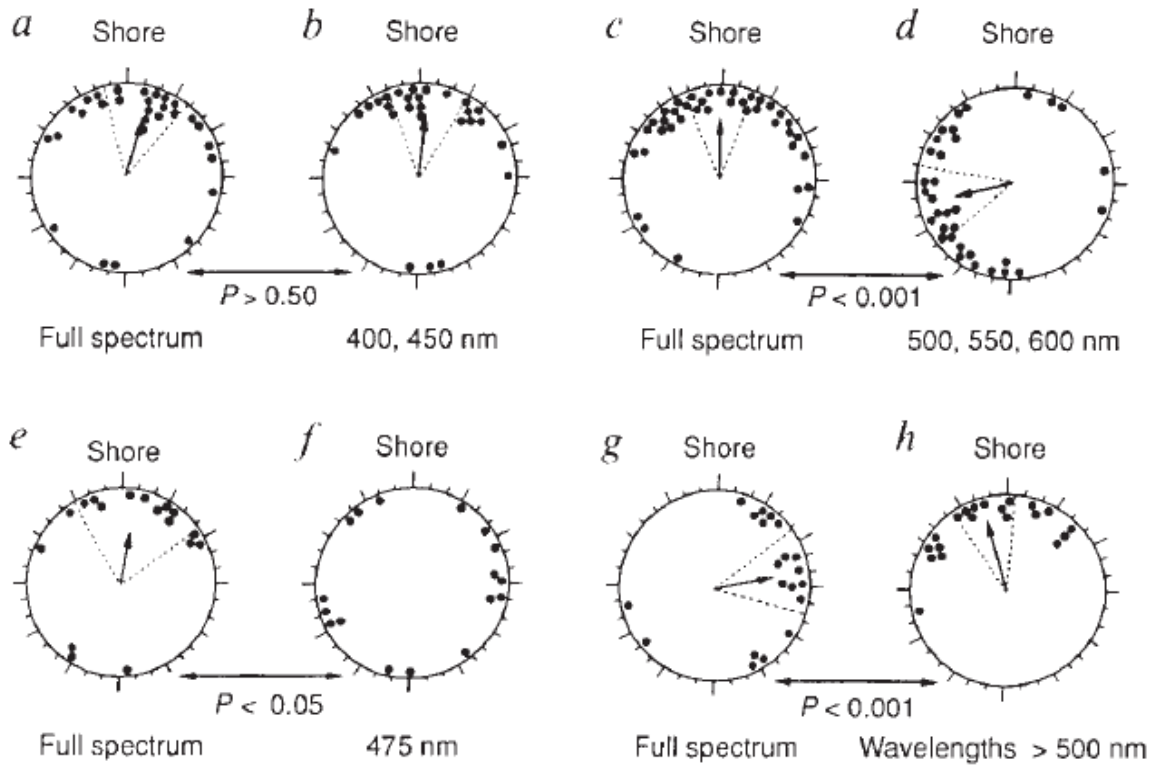


Figure 1.6- (Courtesy of Philips and Borland, 1992 a, figure 1); images a and b demonstrate how the newt's magnetic compass was not affected at low wavelengths (400nm, 450nm); images c and d demonstrate the 90 degree counter-clockwise rotation under long wavelengths (500nm, 550nm, 600nm); images e and f demonstrate an abolishment of the magnetic compass at 475nm which is very similar to the results obtained under >715nm conditions; images g and h are the results from the newts trained using a 500nm filter and demonstrate a 90 degree clockwise rotation when tested under a full spectrum white light.

The effect of light on the magnetic orientation of migratory birds was conducted by Wiltschko and colleagues (1993) on the silvereye species, *Zosterops lateralis*.

Orientation tests were performed under full spectrum white light (approximately 300 nm-700 nm), blue light (443 nm), green light (571 nm), and red light (633nm). The results of this study indicate that the birds orient in the seasonally appropriate direction (NNE) for their species under all wavelengths except red light, where the magnetic compass is abolished.

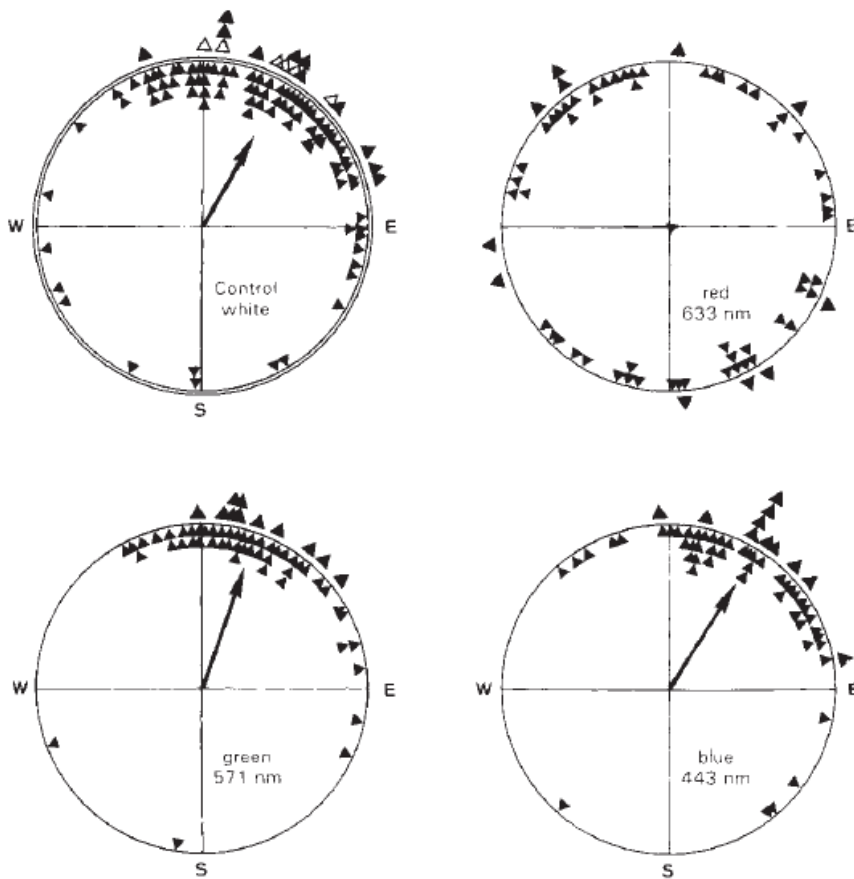


Figure 1.7- (Courtesy of Wiltschko et al., 1993, figure 1); Results of the migration direction under different wavelengths of light demonstrate that the only wavelength to disrupt the magnetic compass was the red light condition; the arrows in each image are representative of the mean vector path for the birds tested.

Cryptochrome-mediated magnetoreception has been suggested to operate through alterations in the spin state of radical pairs (Eveson et al., 2000; Giovani et al., 2003; Maeda et al., 2008; Ritz et al., 2012; Solov'yov and Schulten, 2012). In Giovani and colleagues study (2003) it was demonstrated that light reactions in cryptochrome are mediated through an electron transfer from a tryptophan or tyrosine residue to a flavin adenine dinucleotide cofactor.

1.8 Power Frequency Electromagnetic Fields in The Environment

As the globe shifted from an industrial-driven to a technology-driven economy, the Earth's landscape began to change in ways perhaps not anticipated. For example, using a global average, each person consumes well over three thousand kWh of energy per year. The energy used is delivered at a rate of 50 or 60 Hertz, depending on the country. This power supplies our homes, offices, schools, and everything in them; such as televisions, alarm clocks, fridges, microwaves and computers. As described by Maxwell's equations, which demonstrate the interrelationship between electric and magnetic fields, the consequence is we are constantly inundating ourselves in these magnetic fields.

Furthermore, as telecommunications have rapidly evolved, wireless technologies have introduced a nearly infinite number of uniquely-patterned fields using various parts of the electromagnetic spectrum. These “novel” patterns perfuse into new areas of our lives. Cell phones, laptops, and vehicles ensure we are continuously exposed to a variety of electromagnetic fields. Yet, humans aren't the only species affected. These

same fields are occurring every second of every day and they completely encapsulate the Earth. If many species have demonstrated magnetoreception over a multitude of studies, what are the consequences of the human-made magnetic fields that are now surrounding the planet?

Several studies have reviewed the additional effects of electromagnetic fields on biological systems. These studies have demonstrated much more than a mere orientation effect. For example, Jenrow and colleagues (1995) demonstrated that a significant impairment in regeneration of planarian (*Dugesia tigrina*) following 48 hours of combined exposure to DC (78 μ T) and AC (60 Hz, 10 μ T) magnetic fields. A similar yet much more robust effect was reported from Murugan et al., (2013), where the same species was exposed to two separate fields in a specific configuration over the period of 5 days. Exposure to this sequence produced 100 percent dissolution of planarian, while reversal of sequence or individual field presentations did not produce this effect.

This effect appears to be consistent with other research which has demonstrated that the application of different magnetic field parameters resulted in significant DNA damage in a variety of cell lines (Lai and Singh, 1997; Wolf et al., 2005). A 30 percent increase in HL- 60 leukemia cell proliferation was observed in vitro following 72 hours of applied 50 Hz 1mT magnetic field (Lai and Singh, 1997).

To further highlight the variety of reported effects, field intensities as low as 10 nT have demonstrated significant physiological consequences. In a study using a range of applied intensities of a 7 Hz magnetic field (<1 nT, 1 nT, 5 nT, 10 nT, 50 nT, and 500 nT), a significant increase in brain weight of rats exposed to these fields during their

mother's pregnancy was observed in the 10 nT and 50 nT conditions (Whissell et al., 2008).

Yet, not all results of applied electromagnetic fields have demonstrated damaging effects. Several studies have demonstrated an antinociceptive effect from magnetic field intensities ranging from 1 μ T to 100 μ T (Fleming, Persinger, and Koren, 1994; Thomas et al., 1997; Martin, Koren, and Persinger, 2004). In the latter study, exposure to a 1 μ T magnetic field for 30 minutes resulted in thermal analgesia comparable to 4 mg/kg of morphine in rats. Furthermore, while aforementioned studies have demonstrated impaired regeneration or even dissolution of planarian exposed to electromagnetic fields, Gang and colleagues (2013) demonstrated the opposite effect when using a different patterned field (7 Hz, 100nT) over the course of five nights.

While there is clearly an intensity-dependence for a given response to electromagnetic fields, the pattern of the field appears to be the critical component for the type of effect that will occur. Two studies, one by Walleczek and Lidbury (1990) and the other by Walleczek and Budinger (1992) provide strong support for this rationale. In each study they demonstrated a dose-dependent relationship between intensities ranging from 1.6 to 28 mT, such that no effect was observed in control. The effect began above at 1.6mT. The maximum effect was observed around the 28mT condition. In both studies, calcium uptake was measured. However, the former utilized a 3 Hz field while the latter utilized a 60 Hz field. The 60 Hz condition resulted in enhanced Ca²⁺ uptake, whereas the 3 Hz field produced an inhibitory effect on Ca²⁺ uptake, demonstrating a temporal pattern-dependent response.

Chapter 2: Electromagnetic Effects on the Tunneling Behaviour of the Harvester Ant, *Pogonomyrmex* sp.

2.1 Introduction

Ants are eusocial insects that exhibit an extraordinary complex organization of behaviours that often suggest an operation of a “gestalt” or overlaying field by which the individuals interact with each other and their environments. The role of pheromones, or chemical traces, in the ants’ orientation and spatial movements have been well studied, there are many other environmental variables that can contribute to their behavior. These may range to those as conspicuous as the construction of entrance holes by harvester ants (*Pogonomyrmex occidentalis*) on the south-east side of mounts in order to increase heat levels from the morning sun (Romey, 2002) to the modulating influence of humidity on the foraging activity of the entire ant colony (Gordon et al, 2013). In the latter case the effect of returning foragers upon the rate of exigent foragers increased with humidity.

The potential influences of ambient magnetic fields upon ant behaviour are conceptually intriguing from several perspectives that range from low to high probability mechanisms. Geomagnetic fields with similar probabilities within a locality have the capacity to penetrate millions of ants at the same time and to potentially integrate some aspects of their behaviours. For example assuming 10^5 neurons per ganglia only 10^6 ants are required to match the numbers of neurons within the human cortices. Whereas emergent properties of complex behaviours in the latter are strongly correlated with the intercorrelation of neurons through coherent waves over areas much

larger than the individual neurons, analogous integration might occur among hundreds of thousands of ants connected by magnetic flux lines.

Such interaction would be enhanced potentially if ants displayed magnetoreception. De Oliveira et al., (2010) measured segments of ants head, thorax, and abdomen for Fe/O, Si/Al/O and Fe/Ti/O particles in clusters of 14 μm with particle sizes between $<200\text{ nm}$ and $6\text{ }\mu\text{m}$. Compared with other body parts large concentrations of 50 nm Fe/O particles (and less Si/Al/O particles) were observed in the antennae of ants. Particles surrounding cell-like structures, with semi-major axes between 7.5 and $12.5\text{ }\mu\text{m}$ and semi-minor axes from 3 to $4\text{ }\mu\text{m}$, were frequently found to contain haematite, goethite, and magnetite/maghaemite. The iron-containing areas were observed in the head/scape joint region. The connection of the scape to the head by a ball joint moved by four extrinsic muscles within the cephalic capsule allows a wide range of movement of the antennae.

In addition ultrathin sections of transmission electron microscopy revealed large amounts of 5 to 20 nm diameter Fe/O particles surrounding larger 0.5 to $2\text{ }\mu\text{m}$ particles of Ti/Fe/O or Fe/O. It may be relevant that the two modes of width correspond to the separation between the pre- and post-synaptic junction and the width of the synapse, respectively, in neurons. The presence of ferromagnetic materials with spatial parameters that are similar to neuronal processes that are sensitive to weak, time-varying magnetic fields suggests that ant's may respond to environmental electromagnetic fields at intensities that are typically encountered within the modern environment.

2.2 Materials & Methods

Habitat

Three colonies of Harvester ants (*Pogonomyrmex sp.*) were employed as subjects for this experiment. Each colony was purchased from Ward's science Rochester, New York (Item # 876950) and contained approximately 45 specimens per colony. In order to investigate their tunnelling behavior, colonies were housed in individual Rubbermaid containers measuring 36 X 30 x 15 cm. Each container was modified such that three holes were drilled and a 61 cm piece of 2 cm diameter clear vinyl tubing was inserted into the holes.

Each of the three pieces of vinyl tubing was connected to an individual 15 x 15 x 5 cm compartment, which were subsequently filled with President's Choice black earth extending to the surface container, and placed into a large plastic Rubbermaid container. The soil level was deep enough to cover the three pieces of tubing. This large container had the lid on for the duration of the experiment. The smaller container rested on top of the larger one, such that the tubing ran through the lid into the sealed container. In this way, the top container served as an experimental Earth surface; whereas the three chambers below served as subterranean compartments for the ants to explore. Ants were placed into the surface container at the beginning of the experiment (Figure 2.1).



Figure 2.1 a) Upper left hand corner: schematic of position of the conditions within the colony chamber; b) the tubes for passage within the conditions, c) the top of the colony, d) the soil of the colony.

Electromagnetic apparatus

One of the vinyl tubes served as a control passage whereas the other two tubes had telephone pickups (The Source Barrie, ON; item # 4400533) mounted on the exterior near the entrance. An Arduino uno microcontroller was utilized to generate the electromagnetic signals used for these experiments. One of the signals generated a 60 Hz or fixed pattern EM field. The other generated a patterned EM field. The field patterns were designed off spatial-temporal patterns of fields previously used in biomagnetism studies. The fixed pattern field was the North American power frequency 60 Hertz sine wave. The second has an accelerating phase velocity, which has delays between field presentations that accelerated from 34 ms down to 20 ms at 2 ms increments prior to restarting (Figure 2.2). This pattern is well known to produce analgesic effects in rodents and slowed movement in planarian (Martin and Persinger, 2004; Murugan and Persinger, 2014).

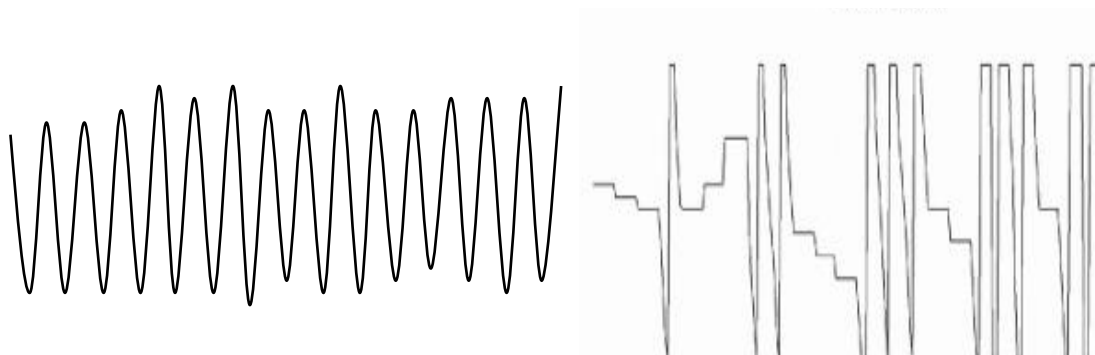


Figure 2.2. Pattern of the 60 Hz fields generated by the Arduino (left) and the classic burst-X pattern (right) known to be associated with analgesia or analgesia like effects in several species.

According to an AlphaLab Inc (www.trifield.com) AC milligauss meter (Model UHS2), the strength of the magnetic fields ranged from 45 mG to 20 mG across the diameter of the “tunnel”. The difference between the 60 Hz and burst-pattern field was approximately a factor of 2 (60 Hz higher). The control tunnel for any given experiment displayed background intensities from ambient power sources of approximately 0.45 mG. Because the control tunnel was about 12 cm away from any of the magnetic field exposed areas, there was no measureable contamination from the applied fields.

2.3 Results

The effects of the two patterns, fixed and phase-accelerating, upon the ants’ movements through the colony space were conspicuous and exhibited an all-or-none response. During the seven days of observation and measurement for all replicates of the procedure the ants avoided the “tunnels” or tubes associated with the proximal applications of the magnetic fields through the commercial solenoids. However the ants freely and frequently utilized the “control” tunnel or tube which changed with each of the replicates. The pictorial results are shown below for various colonies.

Colony A

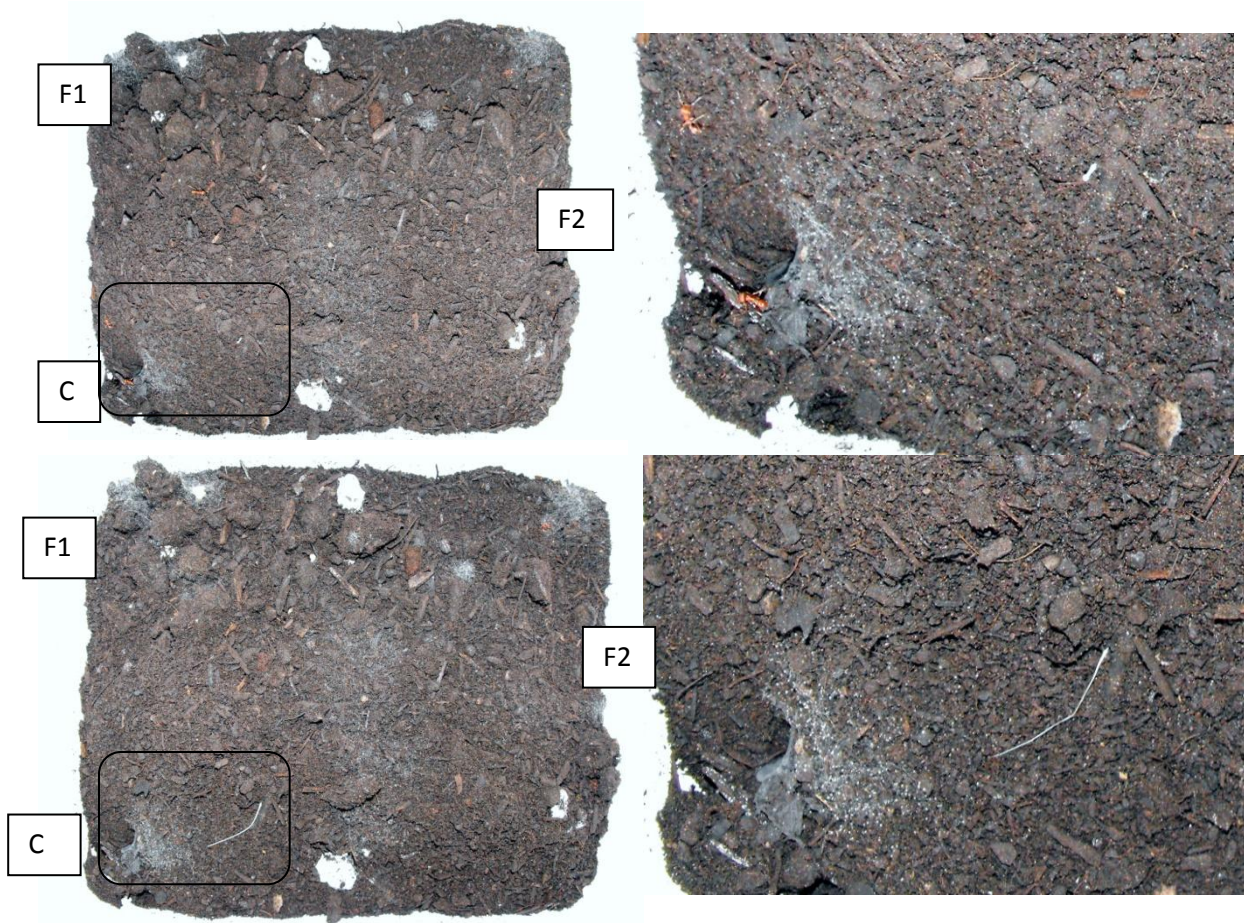


Figure 2.3- Results from colony A; top left= day 1; top right= enlarged image of mound (rounded rectangle in top left) day 1; bottom left= day 7; bottom right= enlarged image of mound (rounded rectangle in bottom left) day 7. Note- Only the control tunnel, surrounded by a rectangle was explored .

Colony B

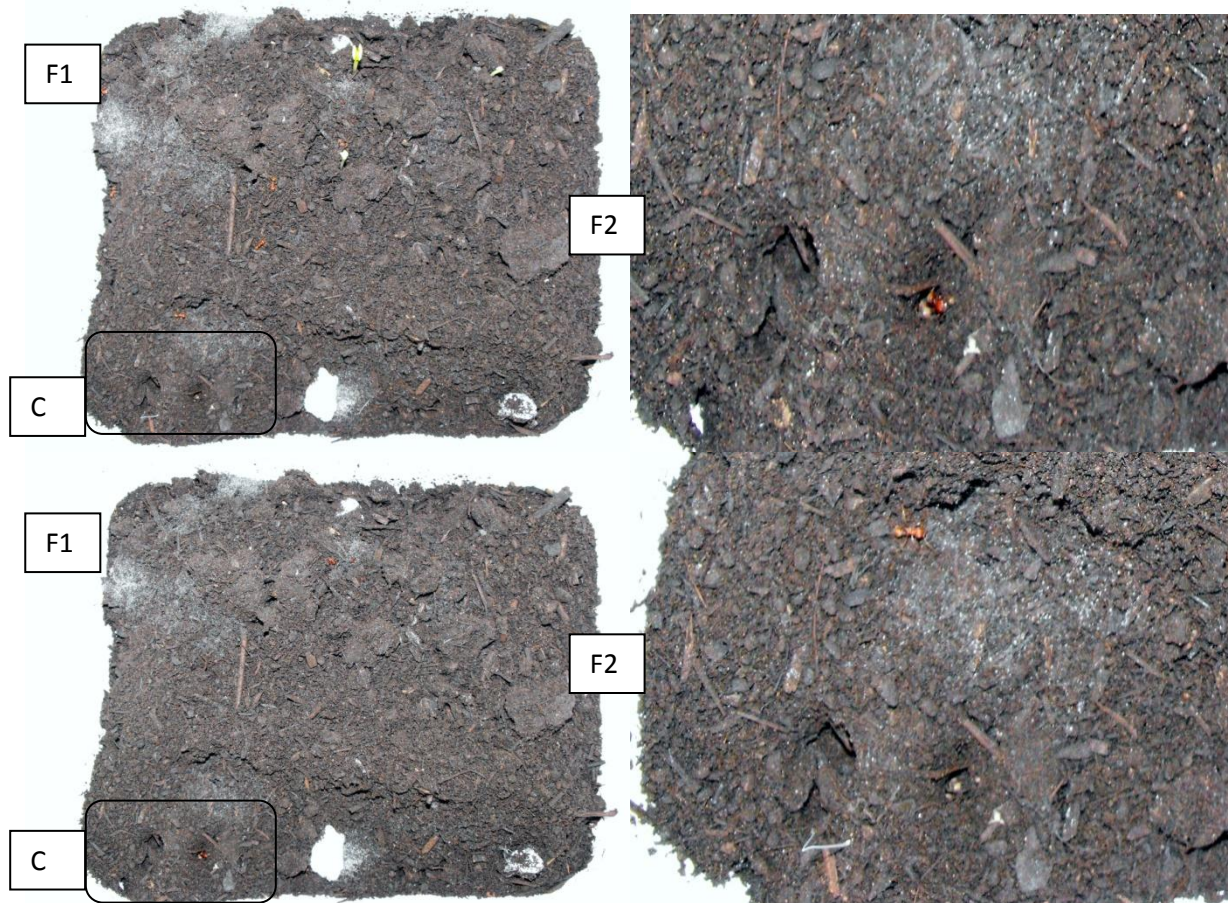


Figure 2.4- Results from colony B; top left= day 1; top right= enlarged image of mound (rounded rectangle in top left) day 1; bottom left= day 7; bottom right= enlarged image of mound (rounded rectangle in bottom left) day 7. Note- Only the control tunnel, surrounded by a rectangle was explored .

Colony C

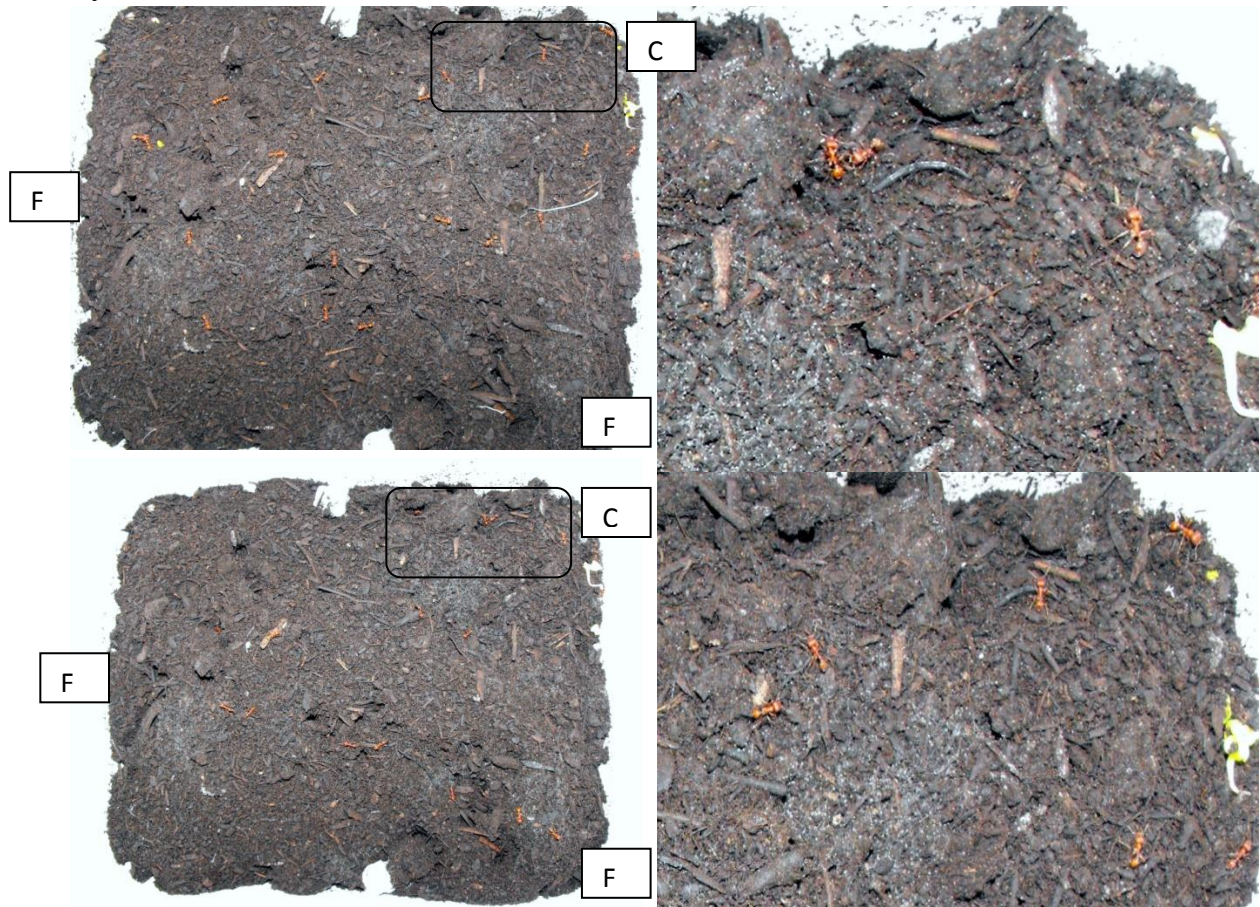


Figure 2.5- Results from colony C; top left= day 1; top right= enlarged image of mound (rounded rectangle in top left) day 1; bottom left= day 7; bottom right= enlarged image of mound (rounded rectangle in bottom left) day 7. Note- Only the control tunnel, surrounded by a rectangle was explored .

2.4 Discussion

The results of these experiments indicated that ants avoid magnetic fields whose intensities are within the 20 to 40 mG (2 to 4 microtesla) intensity range. They avoided both the 60 Hz simulation pattern as well as a pattern that has been known in other

species to induce analgesic-like responses. If avoidance of an area in which a stimulus is presented is defined as aversive, then clearly the stimuli generated from the solenoids were aversive to the ants. There was no evidence of usage of the tunnels near the generation of the magnetic fields.

The ants utilized the tube that served as the “reference” tunnel. The intensity of the ambient magnetic field in this area was about 0.5 mG which was the background levels from ambient power frequencies. Because of the very localized nature of the experimental magnetic fields, their penetration into the control area would have been minimal. If there was an influence the intensity would have been below that of the ambient levels. Even if they were present in sub threshold levels, it is clear their presence did not inhibit the movements of the ants.

Previous researchers have employed field strengths more than 10 times as intense as the ones applied in this study. The application geometry was static presumably to simulate or to cancel the effects of the earth’s magnetic stable magnetic field whose magnitudes are in the order of 5×10^{-5} T. There have also been arguments that magnetic fields stronger than 20 mG are required in order to exert sufficient torque upon the ferromagnetic nanoparticles.

However the energy associated with 10^{-6} Tesla magnetic fields is within range of bioeffectiveness. Torque can be described as:

$$MBv \sin \theta \quad (1),$$

where M is the magnetic moment (A per m), B is the strength of the field, v is the volume and θ is the angle. If we assume the median volume of a cell-like structure

associated with magnetite in the ant antennae to be $1.7 \times 10^{-15} \text{ m}^3$ ($35 \text{ }\mu\text{m}$ by $7\mu\text{m}$ by $7 \text{ }\mu\text{m}$), the intrinsic current is in the order of 1 nanoA from biochemical processes mediated through the cell-like structure to embedded particles, the equivalent “magnetic moment” would be $2.45 \times 10^{-10} \text{ m}^2$ times 10^{-9} A or about 4 A m^2 . The energy from torque assuming the appropriate geometry would be in the range of 10^{-20} J . This is the increment of energy associated with action potentials in mammalian cells as well as the resting membrane potential ($0.7 \times 10^{-1} \text{ V}$ x $1.6 \times 10^{-19} \text{ A s}$).

According to Wajnberg et al (2010) the Ferromagnetic Resonance energy for the magnetite in ants (as measured from the abdomen) is 10^3 J per m^3 . This study also states the magnetite clusters are the aggregation of approximately 10^9 particles, so when we consider the clusters to be $1.7 \times 10^{-15} \text{ m}^3$ (as above), the resultant energy per magnetite particle would be $0.2 \times 10^{-20} \text{ J}$. Obviously the convergence of these measurements do not prove that the ants’ avoidance of the tunnels near these intensity magnetic fields were mediated by the mechanisms and energies just discussed. However it does indicate that the energies generated by the fields within the body of the ants would be sufficient to potentially contribute to their behaviours.

Chapter 3: Macroscopic and Microscopic Effects on the Web Structure of Orb-Weaving Spiders Exposed to Weak-Intensity Patterned Electromagnetic Fields

3.1 Introduction

The contribution of weak, physiologically patterned magnetic fields to the emergence of life and phylogenetic progression has been considered historically by integrative biologists. Burr's *Blueprint of Life* and Pressman's *Electromagnetic Fields and Life* cogently summarize the implicit fact that during the evolution of life processes ambient electric and magnetic fields have been continuous. In addition to the more or less static geomagnetic field there is a wide spectrum of geomagnetic variations which are in the order of a thousand times less intense than the static field. These small changes in geomagnetic activity are correlated with alterations in behavior of species from all phylogeny (Cole and Graf, 1974). The Schumann resonances that range between about 7 Hz and 40 Hz are generated within the earth-ionospheric cavity (Konig et al, 1981). They appear as complex waves or physiologically-patterned magnetic fields that are similar to those associated with cellular processes within invertebrates and vertebrates.

We have been pursuing the concept that complex, physiologically patterned magnetic fields that were likely to have been present since the first amino acids were formed from more massive electrical discharges within primordial atmospheric gases, may be still be particularly resonant with the predominant behaviors that strongly affect an organism's adaptability. For spiders, this primary adaptive characteristic is the

synthesis, formation, and structure of the web. There are different patterns of web configurations that include orb, funnel-type and sheet webs (Rojas, 2011). For the latter different groups of spiders generate different webs that differ in types of silk thread as well as structure (Griswold et al., 2005).

Several studies have shown that web-constructing spiders fed flies containing different psychotropic compounds display altered web formations. Spiders exposed to mescaline, hashish or caffeine (Witt, 1956) or GABA_a (gamma amino butyric) receptor antagonists such as phenobarbital or diazepam (Witt et al, 1968) synthesized markedly different structured webs that different in both geometry and stages of completion. The effects of universal forces, such as gravity upon web-building, as reflected by zero gravity during Skylab experiments, resulted in quantitative changes in web-building that appear to have been confounded by metabolic limits.

Given the sensitivity of web structure to receptor-specific compounds, we reasoned that the appropriate exposure of orb weaving spiders to a complex (physiologically-patterned) magnetic field with intensities known to affect analgesia in other invertebrates such as snails (Thomas et al, 1997) and rodents (Martin et al, 2004), should produce pronounced alterations in web structure. Our working hypothesis is that complex electromagnetic fields that are likely to have been present during the evolution of life forms and hence could exhibit a “biological resonance” may be particularly effective for altering adaptive behaviour. Here we report a robust, qualitatively conspicuous effect of this patterned field upon the web formation and characteristics while exposure to static fields of comparable strengths did not produce these changes.

3.2 Materials & Methods

A total of twelve orb-weaving spiders (*Araneidae sp.*) were collected from Laurentian University campus for the purposes of this experiment. The experiment was conducted on two separate occasions and lasted for a total of five days. Each spider was placed into their own vertical habitat, which were built according to methodologies described by Vogt et al. (2012). Essentially, a sealed transparent plastic container (22 x 22 x 8 cm) was modified with thin string around the periphery to serve as an anchor for the spiders to build their webs.

Each spider was subjected to one of three conditions for the duration of the experiment, two electromagnetic (EM) field conditions and a control condition. The two EM conditions were separated into static and patterned, where the static condition received a 60 hertz field; and the patterned condition received field with an accelerating phase velocity.

The EM fields were presented through a halo (Figure 3.1), a toroidal shaped device that generates a uniform field that surrounds the habitat. The halo was placed in the vertical position in order to be consistent with the manner in which the webs were formed. The toroid's inner distance was 23 cm. It was wound 225 times with 22 gauge speaker wire around a plastic circular form. The field intensities for both presentation types were in the range of 5 to 7 nT, and were generated using an Arduino Uno microcontroller. Because of the weakness of the intensities of the fields, which could still be discerned with an audio amplifier coupled to a telephone sensor jack, the shift in the 3 spatial components of the geomagnetic field was measured by a magnetometer. As

predicted the major shift in the center of the toroid (arrow moving through the center) was 5 to 10 nT when the fields were activated. There was no discernable change in the intensity along the plane parallel with the major axis of the toroid.

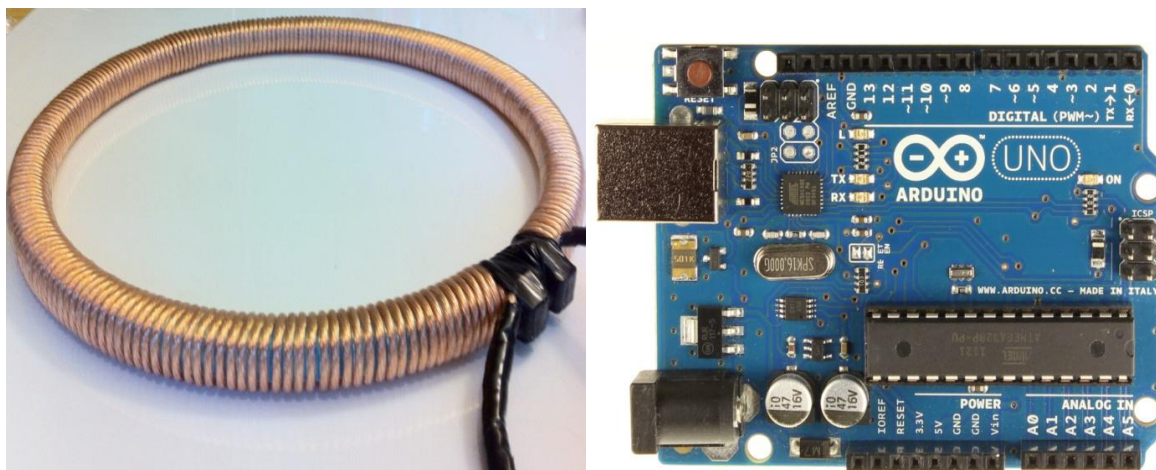


Figure 3.1 - Images of apparatus' used for this experiment. Left-Halo, Right-Arduino Uno microcontroller.

3.3 Results

Following completion of the experiment, samples of the webs formed in each condition were collected and observed under a microscope at 400x. Images were taken using a Fujifilm s1000 10Mp digital camera. The most conspicuous effect for the 8 field exposed spiders compared to the 4 controls (which were exposed to the same equipment except the fields were not generated) was the absence of “glue” droplets on the webs. In addition all of the control or sham-field exposed spiders constructed normal, species-appropriate webs. None of the field exposed spiders displayed appropriate webs. The

strands were visually thinner and were devoid of droplets. Exposure to both the 60 Hz and the burst-firing patterns produced similar qualitative effects. The clear effects are noted in the figures.

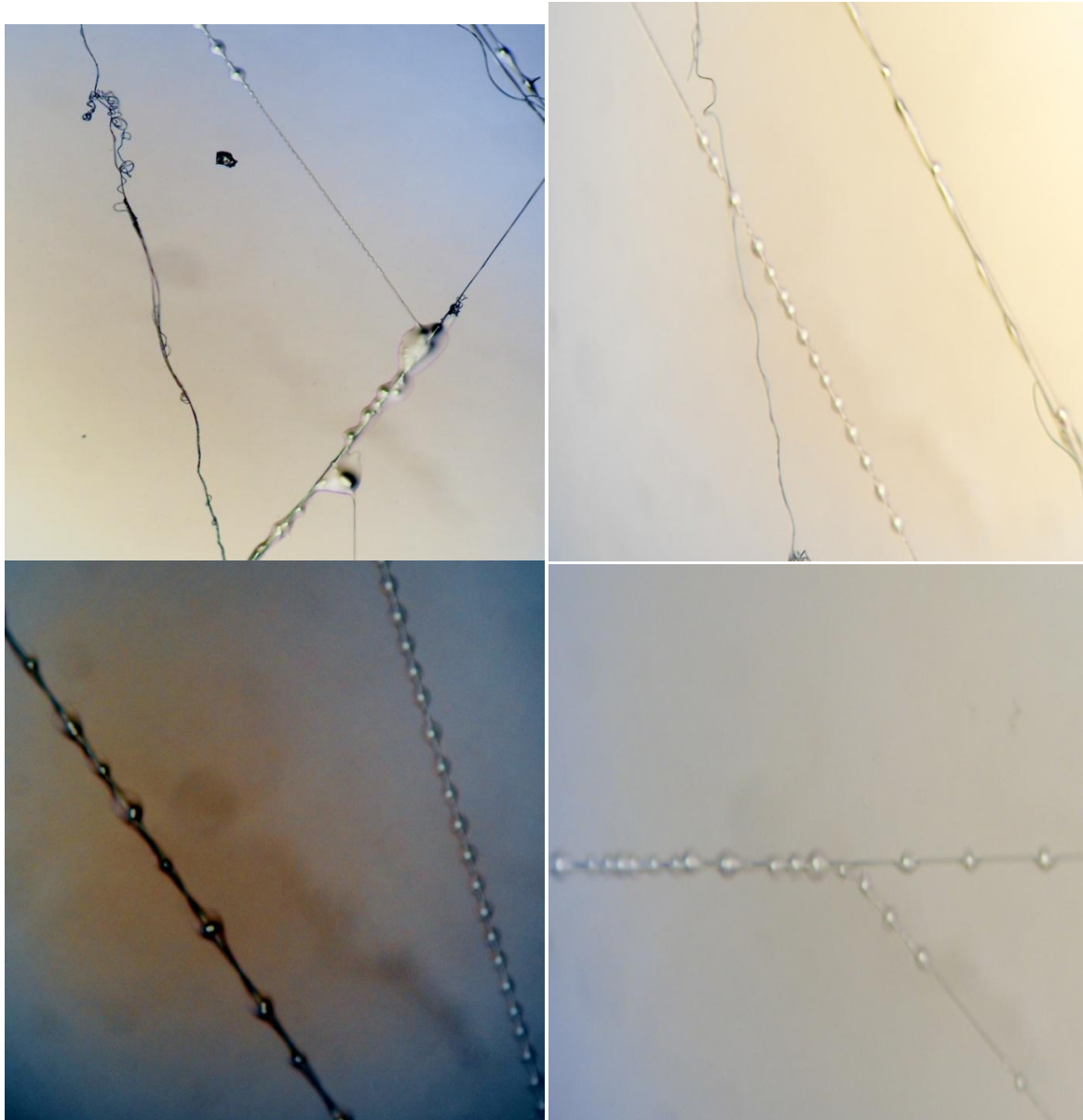


Figure 3.2- Images of the four control samples observed at 400x. Note the presence of the glue droplets.



Figure 3.3- Images of webs of spiders exposed to the 5 to 10 nT toroidal fields observed at 400x. Note the absence of the glue droplets.

3.4 Discussion

The results of these experiments indicated that the exposure to a toroidal magnetic field whose intensities were temporally patterned within the nanotesla range significantly affected web formation as well as the deposition of the viscosity-laden substances upon the web threads. Considering these secretions emerge from special organs that are dependent upon the pH within the microenvironment and their marked dependence upon aqueous reactions, the most likely mechanism by which the applied magnetic fields diminished this process would involve these fundamental processes. Because the patterned (burst) and redundant sine wave (60 Hz) did not produce differential effects, the role of the intensity and spatial configuration of the field may be considered the critical variable. Spiders exposed to the same equipment and toroids but without the activation of the fields behaved as normal spiders.

Direct measurement with a magnetometer indicated that the vertical position of the toroid when activated generated a shifting approximately 5 to 10 nT field through the center of the coil (horizontal to the earth's surface) while there was no change in the stronger vertical component of the local geomagnetic field. Although a shift of 5 to 10 nT may not be considered intense, field strengths of comparable values have been known to facilitate mortality in rat weanlings that have been exposed to electrically unstable physiological conditions such as epileptic seizures (St-Pierre et al., 2007).

There has been theoretical evidence that the toroid as constructed in this experiment may potentially influence gravitational components or some aspect of the space that it occupies. This is suggested by the interesting continuous shifts in the

geomagnetic field within which the toroid was operating such that the local intensities in the plane through the toroid were slowly reduced. Although the more parsimonious explanation is the unique features of the toroid simply cancelled components of the geomagnetic field, the magnitude of the change was very similar to the value that has been associated with the variations in the Newtonian Gravitational Constant (Persinger and St-Pierre, 2014). That spider's respond to "zero" G, at least within the Space Lab, has been suggested by the unusual thin and irregular distribution of radial angles of their webs (Witt et al, 1977).

Witt et al (1977) suggested that the gravitational effect was due to absence of cueing. However there are other possibilities. The gravitational energy within a mass can be described as:

$$J=G \text{ kg}^2/\text{m}^{-1} \text{ (1),}$$

where G is the gravitational constant ($6.67 \times 10^{-11} \text{Nm}^2/\text{kg}^2$), kg is the mass and m is the length. If we assume the mass (Herberstein and Heling, 1999) of the spider is 10 mg ($1 \times 10^{-5} \text{ kg}$) and the length is 5 cm ($5 \times 10^{-2} \text{ m}$), then the energy from that oscillation would be about $1.3 \times 10^{-19} \text{ J}$. This is in the order of a fundamental quantum involved with the movement of protons through water as well as second shell hydrogen values.

The significance of this value is complimented by the magnetic energy produced by the magnetic fields themselves. The energy can be estimated by:

$$J=(B^2/2\mu) v \text{ (2).}$$

Assuming the volume of the average orb spider was about 10^{-8} m^3 , the energy from an average of 5 nT variation through the toroid would be in the order of $1 \times 10^{-19} \text{ J}$.

This convergence with the gravitational energy associated with the spiders' mass could have simulated effects similar to exposure to weightless and hence the subtle contributions from the variations in G .

Chapter 4: Light-Dependent Magnetoreception in Planarian, *Dugesia tigrina*

4.1 Introduction

Planarian are fresh water flat worms who have been popularized by their remarkable ability to regenerate following sectioning from less than one-twentieth of the original mass. They possess a functional nervous system whose characteristics have been described (Okamoto et al, 2005). At least two receptor subtypes for morphine have been identified for planarian (Raffa et al, 2003). In addition two receptor subtypes for melatonin (Dubocovich et al, 2010) suggests these worms are sensitive to ambient photon densities and that these conditions can modulate responses to the same chemical or environmental background.

Murugan and Persinger (2014) reported that planarian exposed to a burst-firing magnetic field for two hours displayed diminished mobility similar to that produced by various concentrations of morphine within their environment. More specifically when planarian were exposed to microMolar to attoMolar concentrations of morphine and to the weak magnetic fields there was an amplification of the effect upon behaviour. There was particular effects produced by two concentrations that were consistent with two receptor subtype binding affinities for morphine. Given the presence of morphine receptors in planarian and their responsiveness to burst-firing magnetic fields, this experiment was designed to discern if these flat worms could differentiate the patterned field from a similar intensity sine-wave field. If the morphine like features of the burst-

firing field were generalizable across phyla then the worms should approach the burst-firing field more frequently than the 60 Hz reference.

4.2 Materials and Methods

Planarian

Two separate experiments were conducted on brown planarian, *Dugesia tigrina*, (Ward's science, Rochester, New York; order number-872500). Ten separate planarian were utilized for each experiment. The first experiment involved the effects of polarity on t-maze navigation. The second experiment tested the effects of the field pattern on t-maze navigation. For each experiment, the planarian navigated a t-maze (placement arm= 3cm x 0.5cm; option arms(2)= 2cm x 1cm) for a total of ten trials each per condition. Six conditions were performed with one condition per day (Figure 4.1, 4.2).

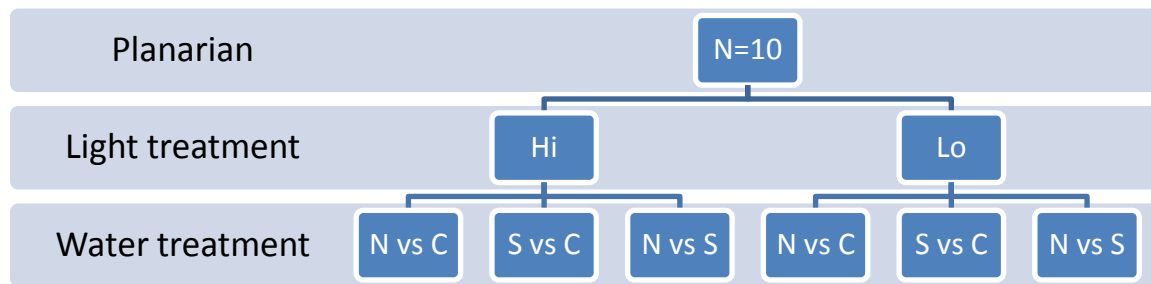


Figure 4.1- Overview of first experimental design. Light treatment was approximately 500 lux for the bright (Hi) condition and approximately 10 lux for the dark (Lo) condition. Water treatment refers to the magnetic exposure at the end of either of the arm selections on the t-maze. [N=north pole; S=south pole; C=control]

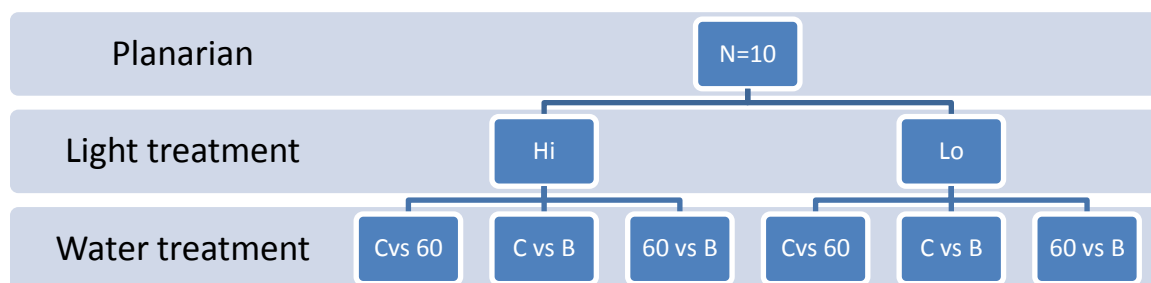


Figure 4.2- Overview of second experimental design. Light treatment was approximately 500 lux for the bright (Hi) condition and approximately 10 lux for the dark (Lo) condition. Water treatment refers to the magnetic exposure at the end of either of the arm selections on the t-maze [C= control; 60= sixty hertz field; B= Burst field]

Light treatment

In order to discern whether magnetoreception may be influenced by differing light conditions, “hi” (bright) and “lo” (dim) light conditions were used. In the hi light condition, planarian navigated the maze in a brightly fluorescent-lit room (about 500 lux) such that no shade was available at any point in the maze. The dim light condition was performed in near-dark (between 1 and 10 lux), such that the planarian navigation was barely visible.

Magnetic Field Procedures

For the first experiment, small coin magnets (2 cm x 1 cm) were placed beneath the arms of the t-maze, either north pole facing up, the south pole facing up, or a plastic replica. In this manner, potential polarity effects could be investigated during t-maze trials. For control conditions, a dark piece of plastic with similar dimensions to the magnet was substituted.

For the second experiment, small solenoids (2cm x 4cm) were placed in a similar location as the coin magnets for the previous experiment. For the control conditions, the solenoids were still utilized; however, no electrical power was provided. An Arduino uno microcontroller was utilized to generate the electromagnetic signals used for these experiments. One of the signals generated a 60 Hz EM field, while the other generated a patterned EM field (BurstX) having an accelerating phase velocity, with delays between

field presentations from 34 ms down to 20 ms at 2 ms increments prior to restarting (Figure 4.3).

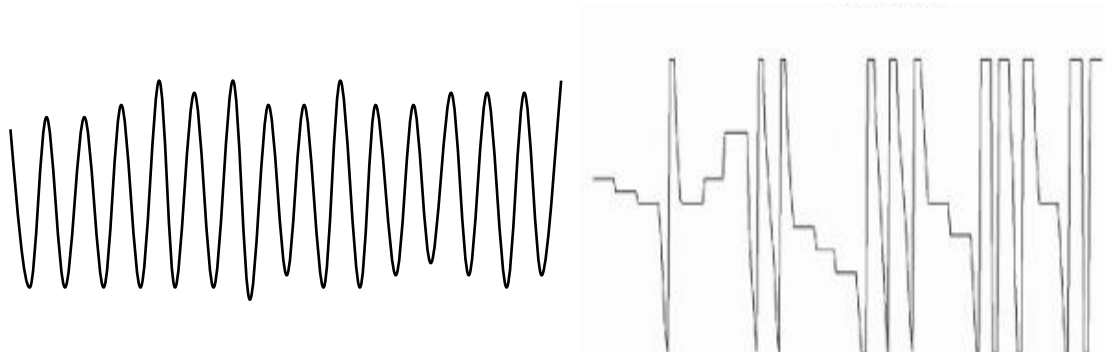


Figure 4.3 Magnetic Field Intensities and Configurations

According to a power meter (Milligauss, AlphaLab), the strength of the magnetic fields from telephone solenoids at the end of each arm was about 25 mG. At the distance of the interface with the first component of the maze, where the response to move to either the 60 Hz or burst-x pattern would have occurred (3 cm away from either field), the intensity was about 1 mG. In comparison the field strength and the beginning of the T-maze was similar to background (about 0.5 mG).

For the coin magnets magnetometer measurements were completed. Figure 4.4 presents average intensity's for each component of the magnetic field.

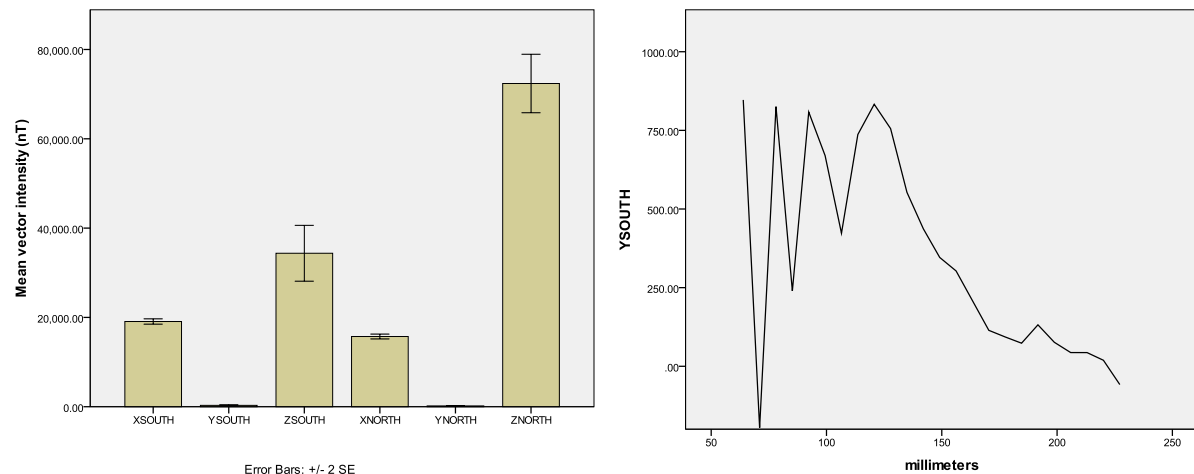


Figure 4.4 Mean (Resultant) intensity within the maze produced by the coin magnets (left). The change in intensity as a function of distance from the coin magnets (right).

Figure 4.5 depicts the x, y, and z components (nT) as a function of distance (mm), including measurements when the north pole faced up and those when the south pole faced up. Although the most drastic changes in intensity as a function of distance were observed for the x and the z components, the y-component had the most variability as a function of distance.

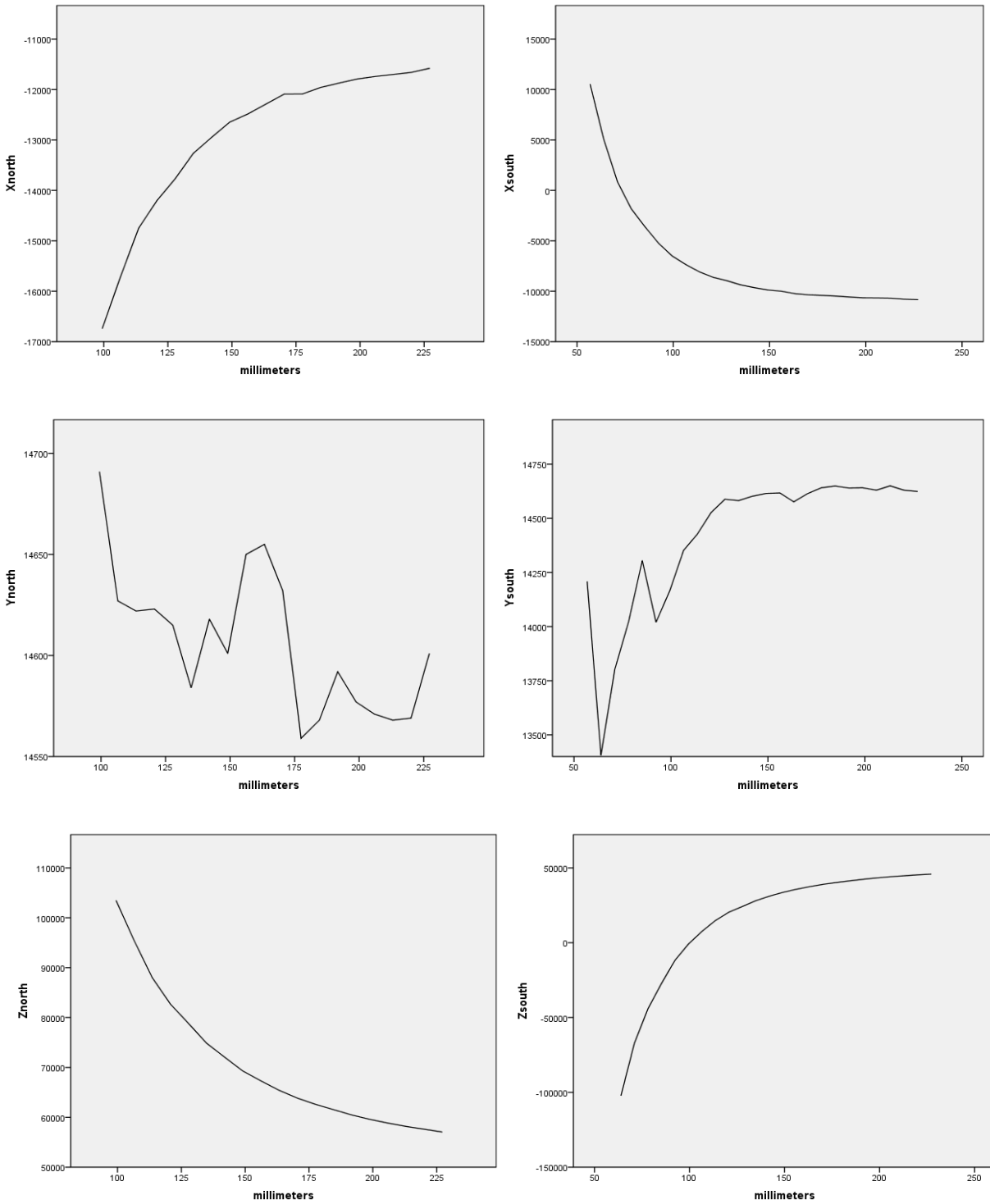


Figure 4.5 Various intensities of the magnetic field strengths (nanotesla) produced by the coin magnets in various spatial planes (mm).

4.3 Results

Polarity

When comparing the control vs. the south polarity group in the bright light condition, planarian moved to the south polarized arm [S $X=7.8$; $SE=0.29$] significantly more times than the control arm [C $X=2.2$; $SE=0.29$] [$t(9)=-9.64$, $p<0.001$, $R^2=0.92$]. A similar effect was observed in the dim light condition, where the south arm [S $X=8.3$; $SE=0.40$] was “selected” significantly more times than the control arm [C $X=1.7$; $SE=0.40$] [$t(9)=-8.34$, $p<0.001$, $R^2=0.89$]. This is shown in Figure 4.6.

When comparing the control vs. north groups in the bright condition planarian were observed to navigate to the north polarized arm [N $X=7.0$; $SE=0.60$] significantly more than the control arm [C $X=3.0$; $SE=0.60$] [$t(9)=-3.35$, $p=0.008$, $R^2=0.55$]. This effect appeared even more pronounced in the dim light condition where the north arm [N $X=8.7$; $SE=0.34$] was selected significantly more than the control arm [C $X=1.3$; $SE=0.34$] [$t(9)=-11.04$, $p<0.001$, $R^2=0.93$]. These results are shown in Figure 4.7

When comparing the north vs. south polarized groups in the bright light condition, there was no significant difference between the number of times the north [N $X=4.9$; $SE=0.97$] or south polarized arms [S $X=5.1$; $SE=0.97$] were selected [$t(9)=0.10$, $p=0.920$]. However in the dim light condition there were significantly more frequent navigations to the north polarized arm [N $X=7.3$; $SE=0.56$] than to the south polarized arm [S $X=2.7$; $SE=0.56$] [$t(9)=-4.12$, $p=0.003$, $R^2=0.65$]. This is shown in Figure 4.8.

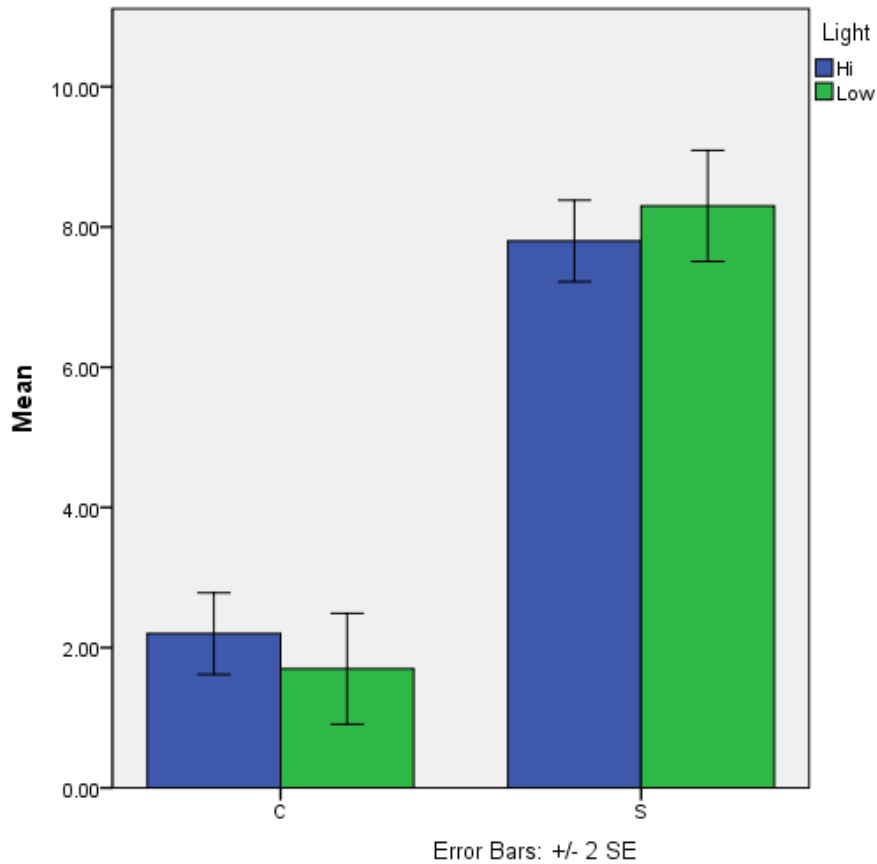


Figure 4.6- Results from the planarian trials for the control versus south polarized arms, for both the high and low light conditions. The planarian in both hi and lo light conditions entered the south polarized arm significantly more than the control arm. On the x-axis, C=control and S=south polarized; and the y-axis the values indicate the mean number of arm selections.

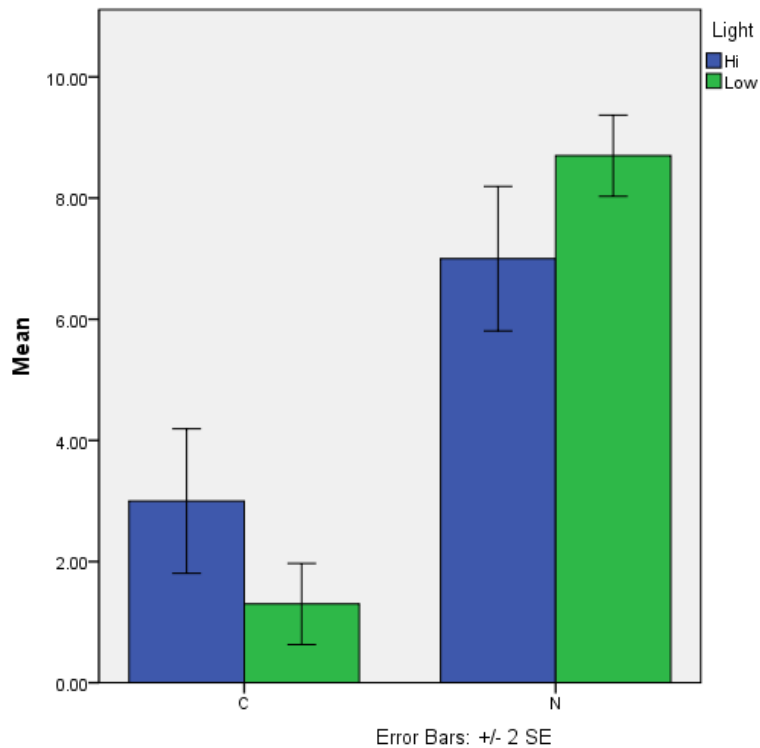


Figure 4.7- Results from the planarian trials for the control versus north polarized arms, for both the high and low light conditions. The planarian in both high and low light conditions entered the north polarized arm significantly more than the control arm. On the x-axis, C=control and N=north polarized; and the y-axis the values indicate the mean number of arm selections.

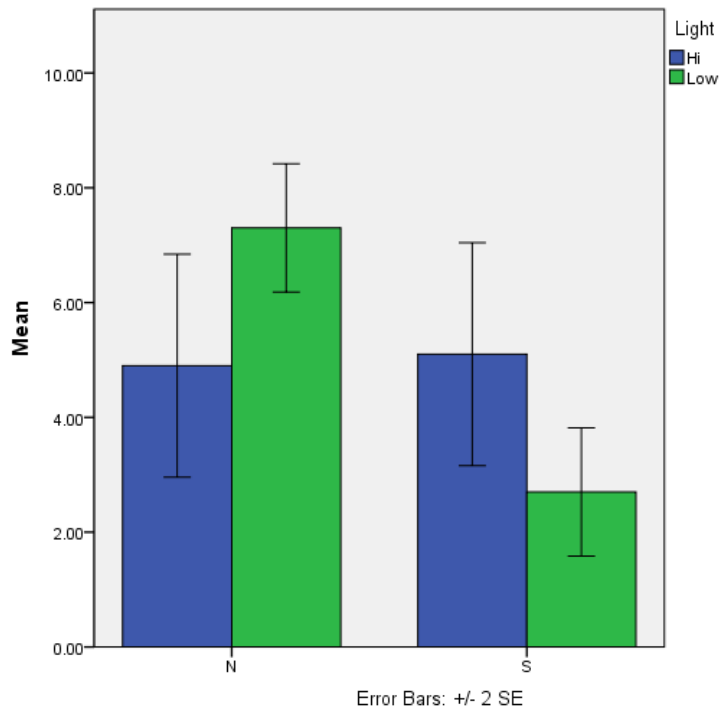


Figure 4.8- Results from the planarian trials for the north versus south polarized arms, for both the high and low light conditions. The planarian in the hi light condition did not display a bias for either the north or south polarized arms; however, in the lo light condition the north polarized arm was entered significantly more than the south polarized arm. On the x-axis, N=north and S=south polarized; and the y-axis the values indicate the mean number of arm selections.

Selection of the Pattern of the Field

When planarian were tested when one arm of the maze generated the 60 Hz field and the other arm generated the burst-firing field there were significantly more movements to the burst-firing arm. More specifically when comparing the control vs.

60 Hz exposed groups in the bright light condition, the 60 Hz exposed arm was entered significantly more times [60 X=5.8;SE=0.36] than the control arm [C X=4.2;SE=0.36] [t(9)=-2.23, p=0.05]. This effect was more pronounced, however, in the dim light condition where the 60 Hz exposed arm [60 X=7.1;SE=0.31] was entered significantly more times than the control arm [C X=2.9;SE=0.31] [t(9)=-6.68, p<0.001, R²=0.83]; Figure 4.9.

When comparing the control vs. Burst exposed groups in the bright condition, the Burst field-exposed arm was entered significantly more times [Burst X=6.3;SE=0.40] than the control arm [C X=3.7;SE=0.40] [t(9)=-3.28, p=0.009]. This effect was also more pronounced in the dim light condition where the Burst exposed arm [Burst X=7.9;SE=0.18] was entered significantly more times than the control arm [C X=2.1;SE=0.18] [t(9)=-16.16, p<0.001, R²=0.97]; Figure 4.10.

Finally, when comparing the 60 Hz vs. Burst exposed groups in the bright light condition, the Burst exposed arm was entered significantly more times [Burst X=6.2;SE=0.36] than the 60 Hz exposed arm [60 X=3.8;SE=0.36] [t(9)=-3.34, p=0.009]. As with the other conditions, this effect was more pronounced in the dim light condition where the Burst exposed arm [Burst X=6.9;SE=0.23] was entered significantly more times than the 60 Hz exposed arm [60 X=3.1;SE=0.23] [t(9)=-8.14, p<0.001, R²=0.88]. This is evident in Figure 4.11.

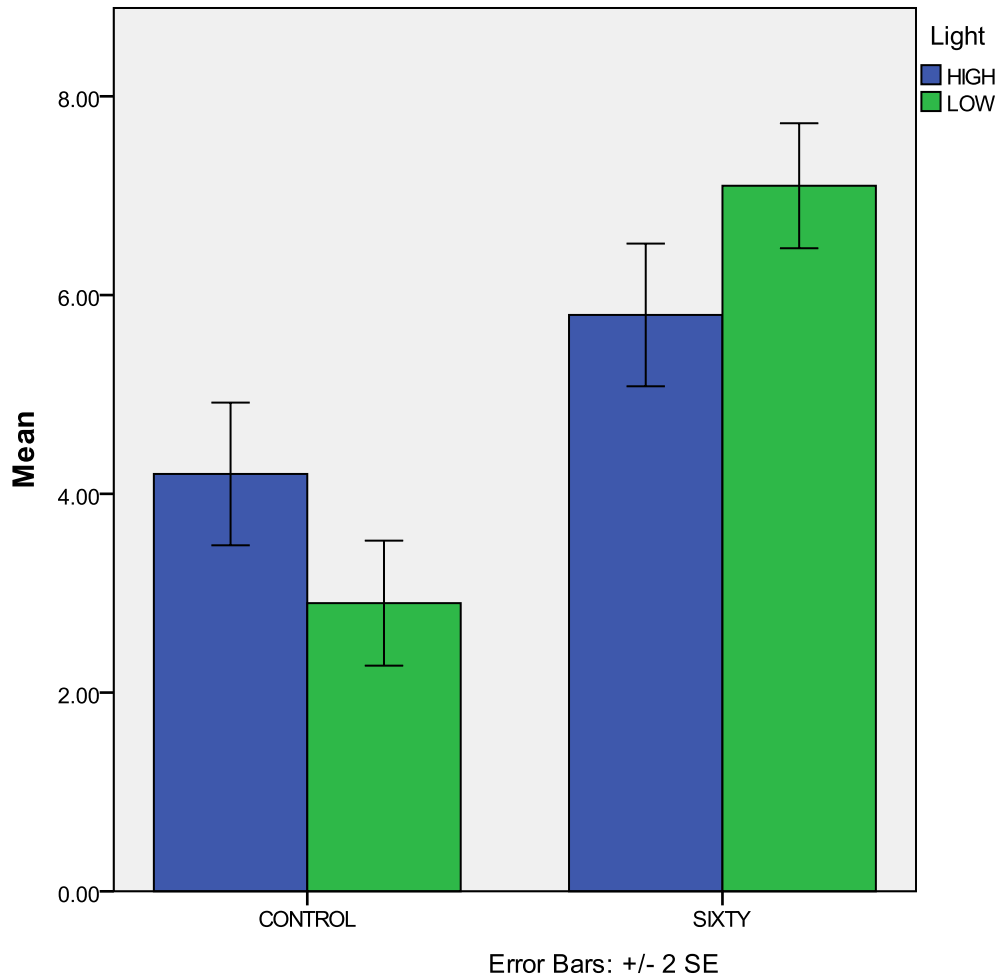


Figure 4.9- Results from the planarian trials for the control versus sixty Hertz exposed arms, for both the high and low light conditions. Arms exposed to a sixty hertz electromagnetic field were entered significantly more than the control arms for both the high and low light conditions.

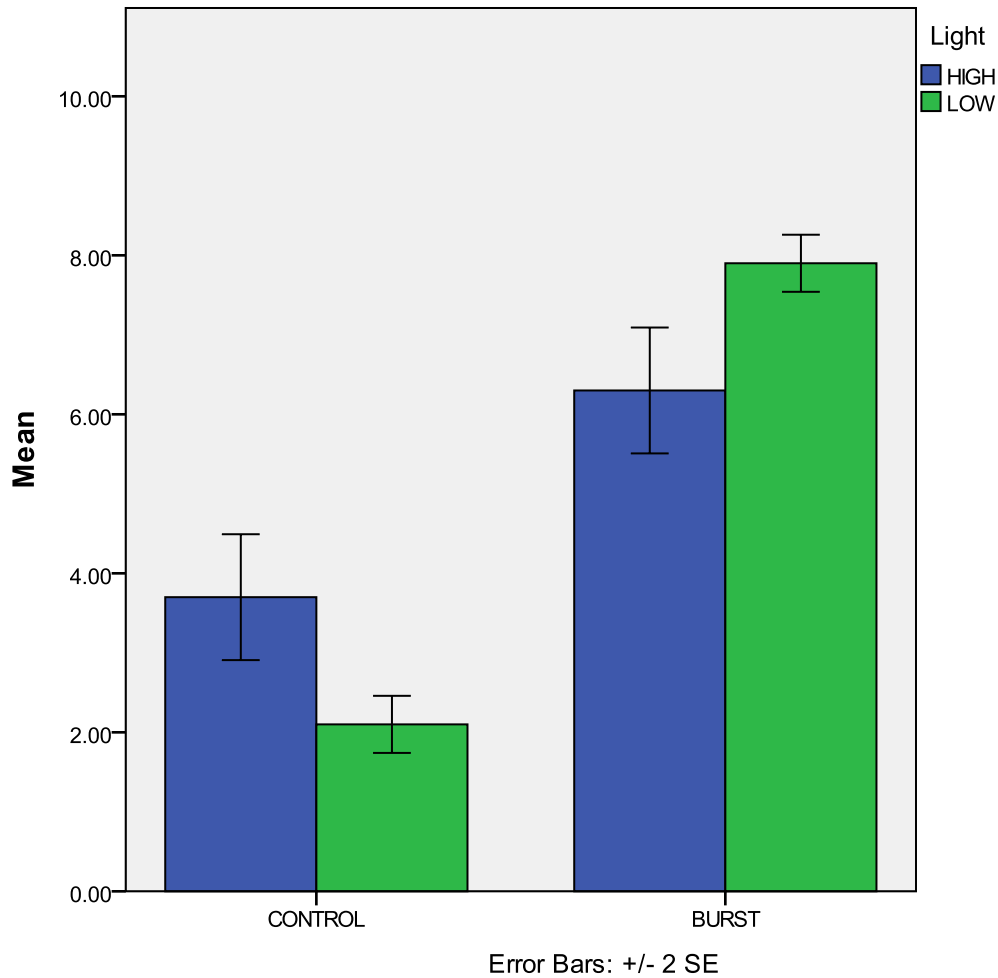


Figure 4.10- Results from the planarian trials for the control versus Burst patterned field exposed arms, for both the high and low light conditions. Arms exposed to a Burst patterned electromagnetic field were entered significantly more than the control arms for both the high and low light conditions.

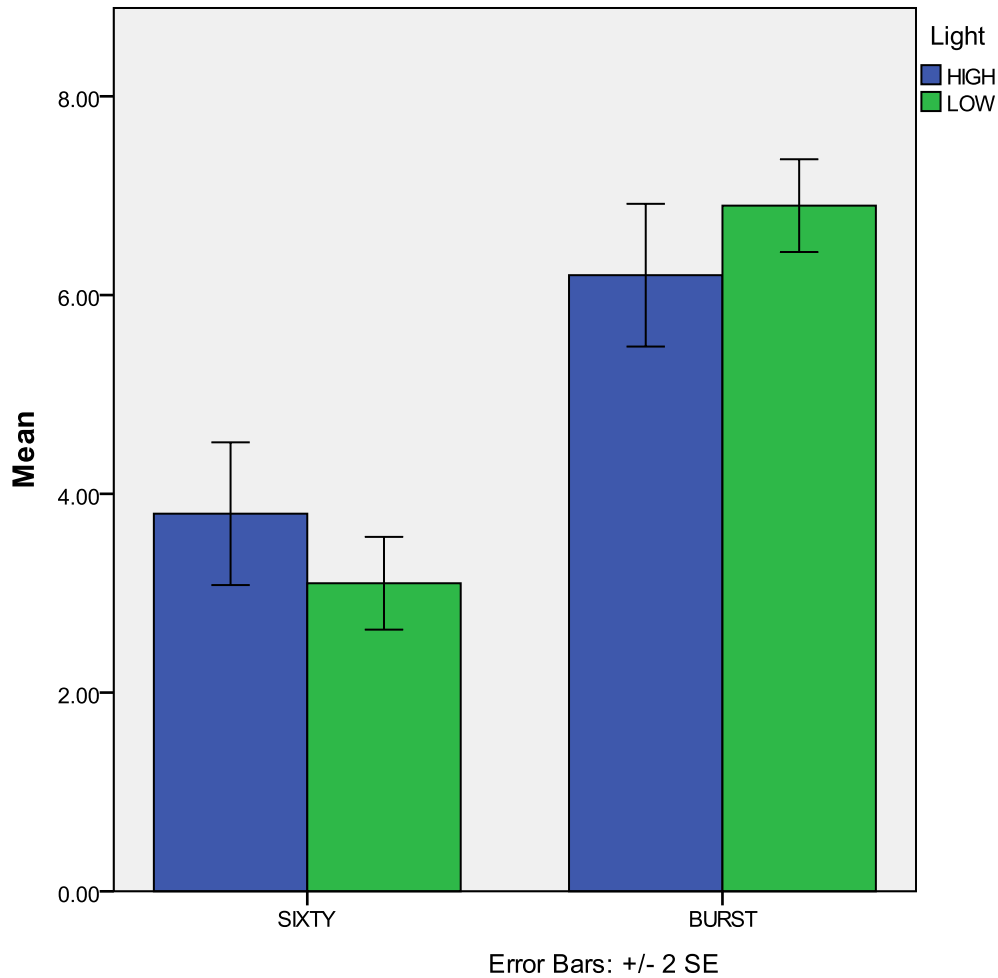


Figure 4.11- Results from the planarian trials for the sixty Hertz versus the Burst patterned field exposed arms, for both the high and low light conditions. Arms exposed to a Burst patterned electromagnetic field were entered significantly more than those exposed to a sixty hertz field for both the high and low light conditions.

4,4 Discussion

The results of these experiments indicate that fresh water flat worms are significantly affected by the polarity of static magnetic fields as well as the pattern of more dynamic magnetic fields. For experiment 1, the planarian responded to the polarity of the experimental fields whose strengths varied from that of the background geomagnetic field to about twice that intensity (100,000 nT). The interaction between polarity and the level of illumination of the background could suggest that photo-like mechanisms or neurochemical pathways are shared by both ambient magnetic fields and photon flux densities. In this instance if the photon levels are too high such as 500 lux or 0.5 Watts per square meter, the magnetic field influence was less.

Even a cursory calculation suggests that the energy from light over the surface area of a planarian given the above photon flux density would be about 10^{-6} J per s. On the other hand the energy from the applied magnetic field at 1 mT for a planarian with a volume of 10^{-8} cubic meters would be 10^{-8} J. Hence the bright light would be more than required to compensate for this energetic effect. On the other hand the same field strength within a dim environment, which would have been at least 100 times less than the bright condition, would have been less than the energy associated with the magnetic field. Hence its effects could more strongly affect the planarian movement.

The second experiment demonstrated that planarian can differentiate between the pattern of two dynamic magnetic fields whose strengths are within the microtesla range. Such intensities are commonly found in the modern environment near electronic

devices such as transformers and large power plants which are often placed near major fresh water sources. In this instance the planarian when exposed to both a 60 Hz sine-wave and a burst-firing pattern known to simulate the effects of morphine, navigated towards the burst-firing field. One might infer that this field pattern is associated with reward features. The enhancement of the effect in dim light would suggest a potentially mediating role of melatonin for which two types of receptors have been measured for planarian.

However it should be emphasized that the planarian were only exposed to these dynamic fields for a few minutes during each trial. The total time would have been around 15 minutes. If the exposure to the burst-firing field had been longer, then slowing of movement similar to an opiate lethargy may have occurred. Murugan and Persinger (2014) found that planarian exposed to this pattern for 2 hrs displayed marked diminished movement that was similar to that produced by the appropriate concentration of morphine that could be attenuated by the opiate blocker naloxone.

Chapter 5: Effects of Static and Patterned Electromagnetic Exposures on the pH of Water

5.1 Introduction

Although water is “the solvent of Life”, its physical properties with respect to the affects of magnetic fields upon biological systems have been rarely studied systematically. Decoursey’s (2003) brilliant review of proton channels within cells and the importance of the hydronium ion (H_3O^+) is perhaps one of the most thorough summaries of the critical properties of this vital substance. Recently, Persinger (2014) showed the quantitative convergence between the physical chemical constants of the proton and the properties of water with respect to their role in weak magnetic field effects both on earth and within galactic contexts.

Gang et al (2012) showed that the physical capacity of water to diffuse a substance was related to the volume of water exposed to a 160 mT static (horseshoe magnet) magnetic field. The duration of the “holding” effect was systematically related to the initial volume of water, either 25 cc, 50 cc or 100 cc, exposed to the magnetic field. Later Murugan et al (2014) found that 50 cc of Spring water exposed to 8 microtesla magnetic fields that were temporally patterned significantly increased the pH of the exposed samples compared to controls. The increase ranged between 0.5 to 1 pH unit (from an initial reading of about pH=7.4) over the approximately 8 hours of exposure. The shifts occurred as micro alterations in the order of a 0.02 pH units whose durations were about 20 to 40 ms.

In the previous chapter planarian exposed to coin magnets with different polarities showed remarkable responses compared to control conditions. The possibility that the effects of the fields upon water itself may have been the primary mediator was considered a hypothesis worthy of pursuit. Consequently the pH of water was monitored over a duration that was similar to that involved with the exposures of the planarian.

5.2 Materials & Methods

In order to monitor the change in pH over time, a digital meter was utilized (DrDAQ USB pH meter, www.picotech.com). Four beakers of water, either 25mL or 50 mL, were monitored for forty minutes. For the first ten minutes, three of the beakers rested on small coin magnets, while the fourth served as a control. Next, the beakers were removed from the magnets. This was repeated another time with ten minutes on the magnets and ten final minutes off (see Table 5.1). The magnets used for this experiment were small ferrite coin magnets (20 mm x 10 mm), which produced a field intensity of 0.05T (500 Gauss).

Time	Condition
0-10 minutes	Beakers placed on magnet
10-20 minutes	Beakers removed from magnet
20-30 minutes	Beakers placed on magnet
30-40 minutes	Beakers removed from magnet

Table 5.1- Order of events for each experimental trial.

pH values were recorded every second for the duration of the experiment, and were subsequently imported into SPSS for analysis. The results were analyzed to discern the differences between polarized and non-polarized water, between north and south poles, and between volumes of water (25 mL and 50 mL).

5.3 Results

Polarized vs. non-polarized water

When analyzing the effects of a permanent magnetic field on the pH of water, it was found that magnetized water (Mean=8.27; SD=0.06) had a significantly higher pH than control water (Mean=7.58; SD=0.11) conditions, [$t(2519)=631.6$, $p<0.001$, $R^2=0.94$]. The results are shown in Figure 5.1.

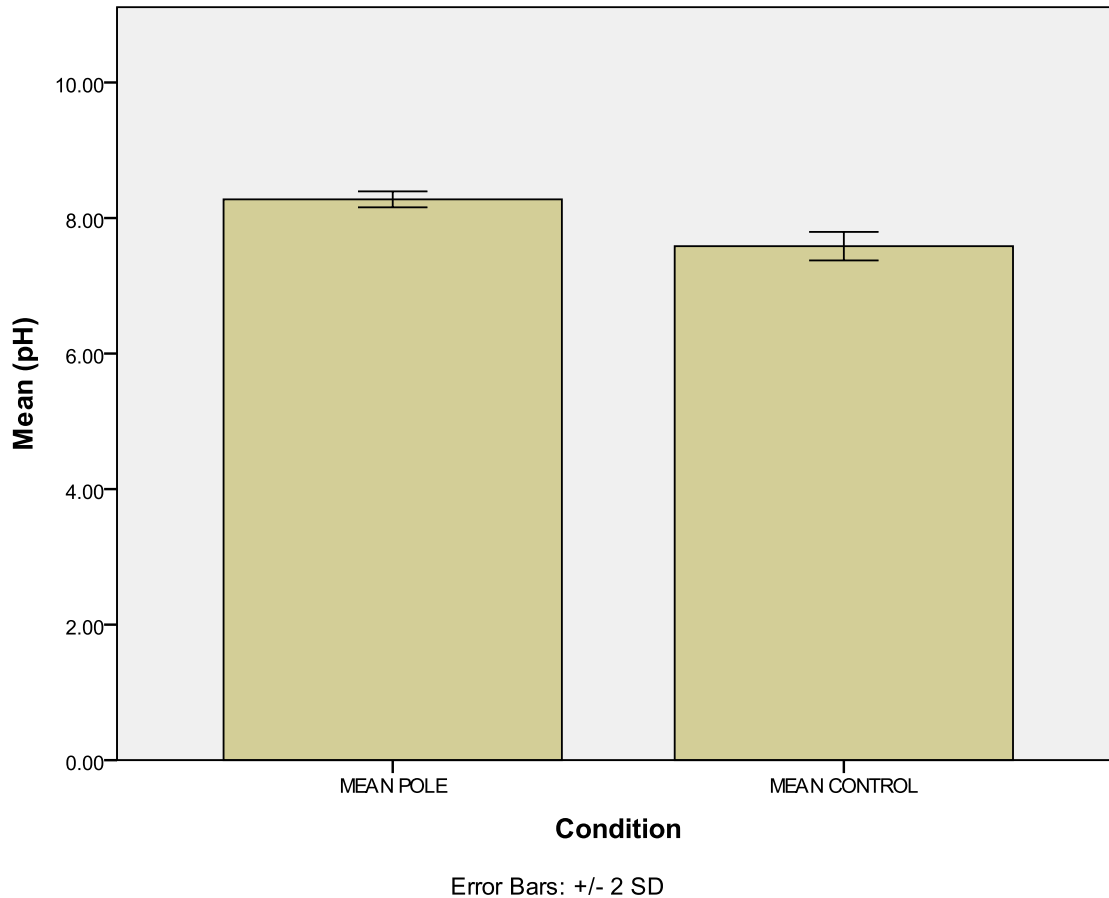


Figure 5.1 - Results from the pH analysis of magnetized water versus control water. The polarized water beakers had a significantly higher pH than the control beakers following the experiment.

North versus south polarized water

When analyzing the effects of opposing poles on the structure of water, north polarized water (Mean=8.42; SD=0.06) had a significantly higher pH than the south polarized water (Mean=8.12; SD=0.07) conditions, $[t(2519)=418.5, p<0.001, R^2=0.84]$. The results are presented in Figure 5.2.

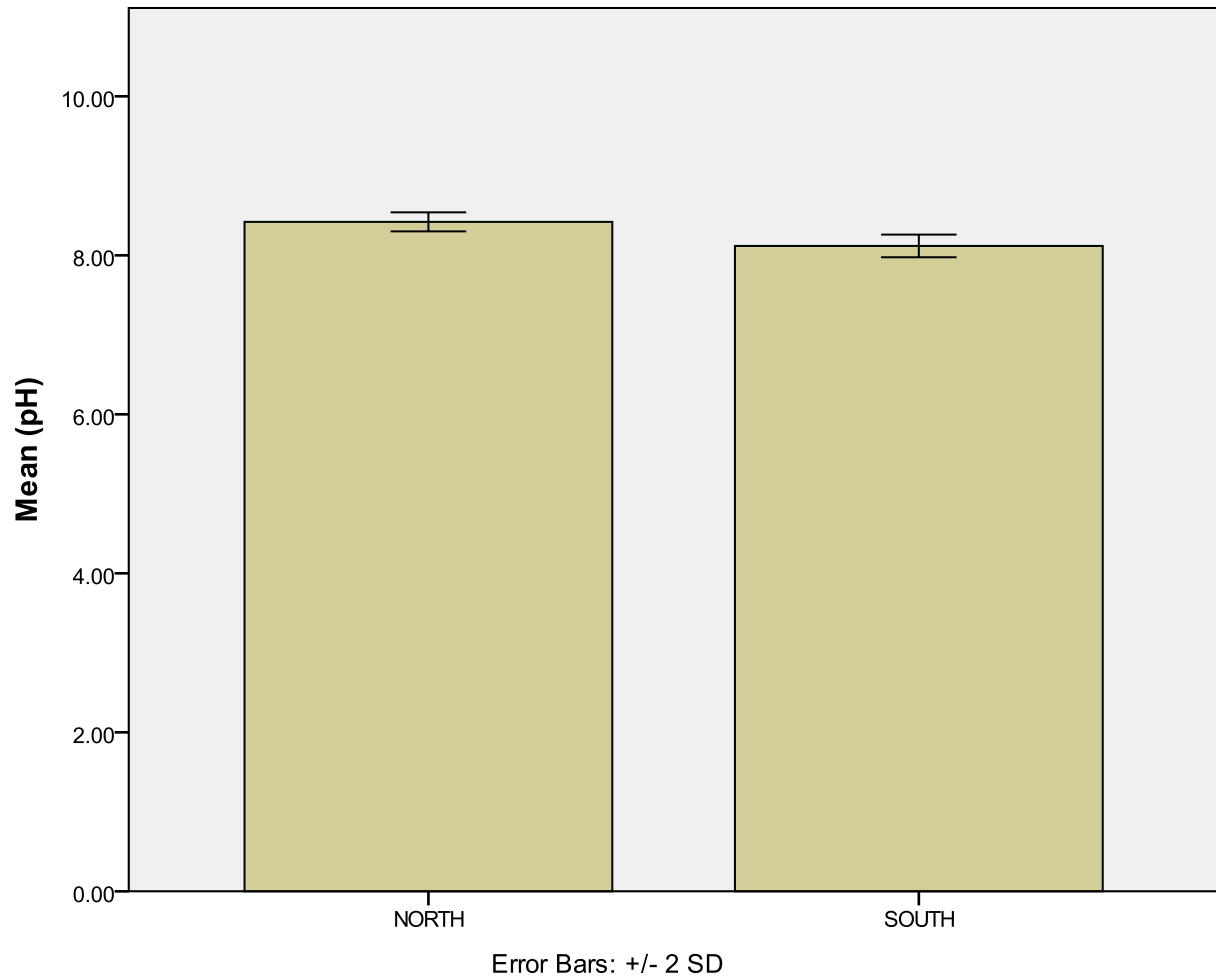


Figure 5.2- Results from the pH analysis of north polarized water versus south polarized water. The north polarized water beakers had a significantly higher pH than the south polarized beakers following the experiment.

Volume: 25 mL versus 50 mL by condition

When investigating the above effects on differing volumes of water, it was found that the north polarized water had a significantly lower pH in the 50 mL condition

compared to the 25 mL condition [Mean=-0.42; SD=0.08; $t(2519) = -259.69$; $p < 0.001$, $R^2 = 0.96$]. A similar effect where the 50mL condition had a significantly lower pH than the 25 mL condition was evident for the south polarized water [Mean= -0.45; SD=0.11; $t(2519) = -203.97$; $p < 0.001$, $R^2 = 0.94$] and the control water [Mean=-0.25; SD=0.17; $t(2519) = -73.20$; $p < 0.001$; $R^2 = 0.68$]. The results are shown in Figure 5.3.

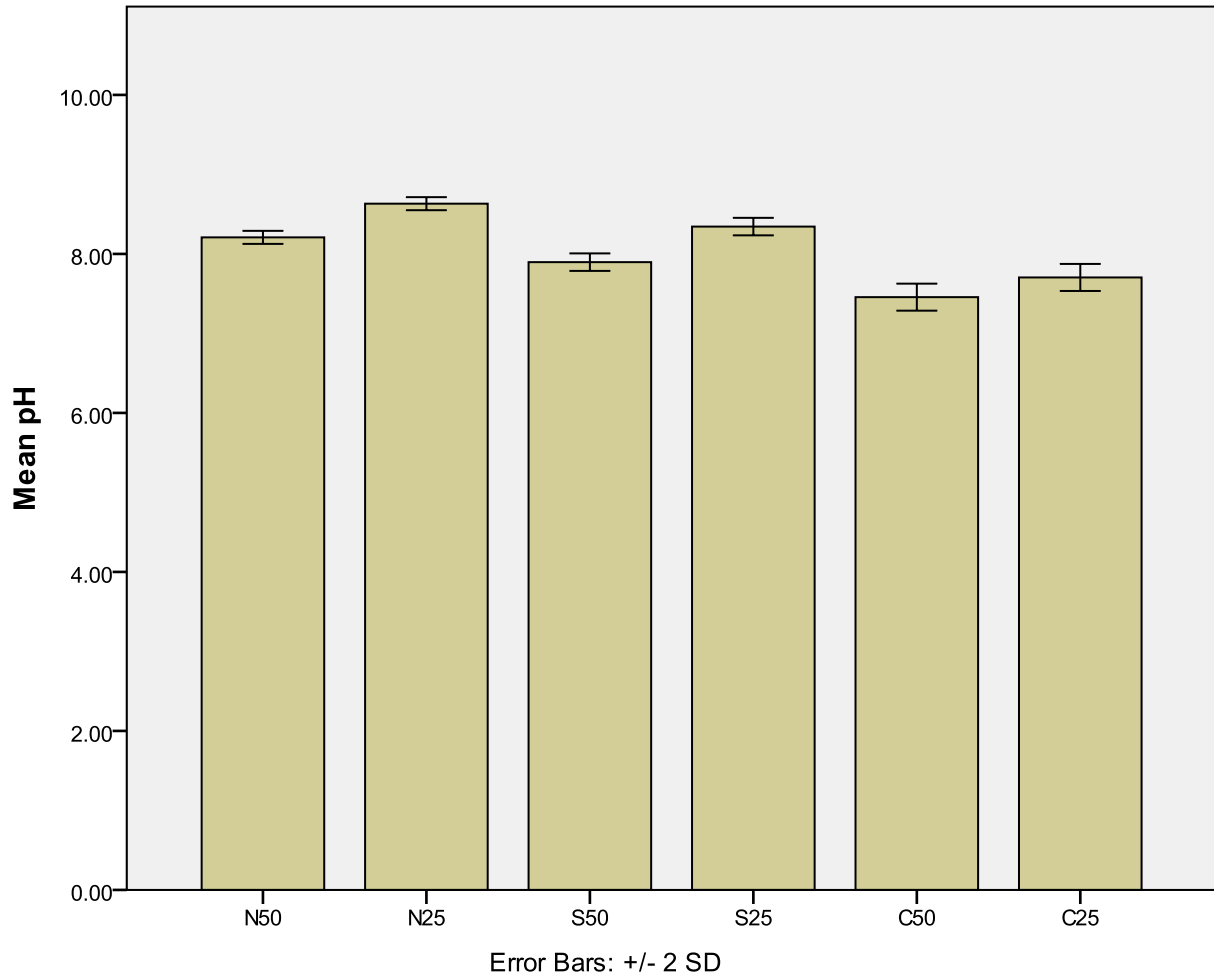


Figure 5.3- Results from the comparing the effects of polarization and volume on the pH of water. (N=north; S=south; C=control; 50=50mL; 25=25mL). The greatest deviation from the control occurs at lower volumes of water.

5.4 Discussion

These results compliment and extend those reported by Murugan et al (2014) that showed a shift towards basic when Spring water was exposed to microtesla, physiologically patterned magnetic fields. In those experiments approximately 8 hours were required for a shift of between 0.5 and 1 pH unit towards alkalinity. In the present study the water exposed to the static (coin magnets) exhibited an increase of 0.7 pH units relative to control water after only 20 min of exposure. The strength of the field was 500 Gauss (0.05 T) or about 1000 times more intense than those employed by Murugan et al (2014). The strength of the field was more similar to that employed by Gang et al (2012) although they did not measure pH directly.

The difference between the north-polarity and south-polarity exposed water was about 0.3 pH units. This could be relevant to the planarian studies in the previous chapter because the north polarity fields were more effective for influencing planarian navigation than southern polarity. If movement of planarian is associated with greater metabolism and hence greater acidity or proton concentration within the worm's volume, then movement towards a slightly alkaline environment may have facilitated the movement of protons into the extracellular and extra-organism environment.

The differential effects of the volumes of the water that were exposed to the polarities is significant. The diminishment of the north polarity effect within the 50 cc compared to 25 cc volumes would suggest that the physical effect associated with the exposure coin magnets resulted in a fixed quantity. When exposed to a larger volume, the impact of this effect was diluted.

CHAPTER 6: General Discussion

6.1 Discussion

This research was intended to investigate potential cross-phyla behavioural effects associated with the application of weak-intensity electromagnetic fields. Considering humanity's rapidly expanding electromagnetic footprint, field geometries and intensities we're selected to match field patterns produced by nearly every home and building in many countries including Canada and the USA . The experiments were designed to add to the growing body of literature highlighting human's electromagnetic effect on the behaviour of various species. Evidence has been provided demonstrating effects dependent on intensity, pattern, light and polarity. These factors will be evaluated for their role in magnetoreception among species.

6.2 Role of pattern and intensity in electromagnetic effects

Taken as a whole, behavioural effects were observed in these studies resulting from exposure to electromagnetic field intensities ranging from 5 nanotesla up to 4 microtesla. One of the most interesting findings in these experiments was the apparent difference when comparing the electromagnetic effects on the harvester ants and orb-weaving spiders versus those observed in the planarian study.

The response appeared to be aversive to some degree in the ants and spiders, where an all-or-none response was noted. For example, the colonies of ants in each

experiment all avoided tunneling near the electromagnetic exposure site, and for the spiders none of the field-exposed spiders completed a web. This effect was contrasted with the planarian, where field-exposed arms of the t-maze were entered significantly more than the control arms.

A second difference between the ants and spiders versus the planarian was the pattern-dependent effect. Whereas, the field exposure produced an aversive response independent of the pattern of the field with the ants tunneling behaviour and the spiders web formation, a clear pattern-dependent response was observed for the planarian, which entered the BurstX exposed arms significantly more than those exposed to 60Hz or controls. The difference between the observed electromagnetic effects among species may suggest different mechanisms at work.

6.3 Light-mediated effects observed in the planarian

The light-dependent effects observed in the planarian investigation may provide additional evidence suggesting different pathways involved in magnetoreception for this species compared with the harvester ants and the orb-weaving spiders. The all-or-none response observed in the ants and spiders is typical of a magnetite dependent type of reception, such that the presence of a field produces a response that is not based on pattern. This pathway is most easily recognized in the work on magnetotactic bacteria (Blakemore, 1975).

The light-dependent mechanism observed in the planarian is more consistent with cryptochrome based magnetoreception. (Philips and Borland, 1992; Wiltshko et al,

1993) demonstrated this type of reaction is sensitive to very discrete wavelengths. In other words, specific waveforms or patterns can be differentiated from others and produce an effect. This specificity is not observed in magnetite dependent magnetoreception giving further support that it wasn't mediated through this type of mechanism.

6.4 Polarity-dependent effects

In addition to investigating pattern and intensity-dependent electromagnetic effects in planarian, polarity effects on t-maze navigation were also analyzed. Similar to the different bacterial responses to polarity observed in bacteria in the north and south hemispheres observed in several studies (Blakemore, 1975; Moench & Konetzka, 1978; Blakemore & Frankel, 1981), the planarian used for this experiment, which naturally reside in the northern hemisphere, entered the north polarized arms significantly more than the south polarized arms or the control arms.

The above results prompted an investigation into an alternate mechanism by which the magnetic fields exerted their effects in planarian. Being an aquatic creature, a change in pH could potentially act as useful information for determination of direction of travel for this species. The pH in the north exposed beakers was on average a full pH unit greater than the control beakers, and roughly a third of a pH unit greater than the south exposed beakers. These results produced similar effects to an 8 microtesla electromagnetic field utilized in the experiment by Murugan et al., 2014; however, in that study changes in 0.5-1.0 pH units were observed following an eight hour exposure,

whereas similar changes occurred in the current study following only forty minutes exposure to the coin magnets.

6.5 Future research

Although these studies have investigated electromagnetic effects across various species, they serve only as a starting point for each. In order to fully know the spatial and temporal limits to magnetoreception in the harvester ant, orb-weaving spider, brown planarian, and water, much more research would be required. In addition to experimental settings, observations of each species in their natural habitat would be ideal to determine approximate population density as a function of distance away from predefined electromagnetic sources. Finally, to complement the macroscopic observations, biochemical analyses would be essential for determining and quantifying a mechanism in any of the species investigated.

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